Studies on Entomopathogenic Nematodes and their role as Bioagents for the Management of Root-knot Nematode *(Meloidogyne incognita)* Infecting Brinjal

DISSERTATION

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IN

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BY

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Dedicated
To

My Beloved Parents

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And
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CERTIFICATE

This is to certify that *Mr.* Javaid Ahmad Lone has worked in this department as a research scholar under my supervision and guidance. His work on the "Studies on Entomopathogenic Nematodes and their role as Bioagents for the Management of Root-knot Nematode (*Meloidogyne incognita*) infecting Brinjal" is up to date and original. He is allowed to submit his dissertation for the consideration of the award for the degree of *Master of Philosophy in Botany*.

(Dr. Tabreiz Ahmad Khan)
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Javaid

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INTRODUCTION

Vegetables are considered one of the largest constituents of the human diet although proportions may vary in different countries of the world. In most of the under-developed countries, majority of population depends entirely upon vegetables. Brinjal is one of the important vegetable crops among them.

Solanum melongena L. commonly known as brinjal/egg plant /aubergine is an important vegetable crop in many tropical and subtropical countries. The name brinjal is popular in Indian subcontinent and is derived from Arabic and Sanskrit whereas, the name egg plant has been derived from the shape of the fruit of some varieties, which are white and resemble in shape to chicken egg. It is also called aubergine (French word) in Europe.

Egg plant belongs to the family Solanaceae and is cultivated throughout India. In India brinjal is grown thrice during the year, therefore, as a vegetable is available almost throughout the year. Considering the production per hectare, the yield is not sufficient to fulfill the demands of the masses. It is self pollinated crop and therefore, genetic variability is limited.

Besides being used as vegetable, brinjals are consumed in a variety of ways, roasted in hot ashes, mashed and seasoned with salt, onion, chilies, lime juice and mustard oil, they are made in to bharta a preparation relished all over India. The brinjal may also be pickled. Sliced fruits are sometimes dried in the sun and stored.

The value of brinjal enhances as a vegetable during autumn when other vegetables become rare. They are eaten when approaching ripeness and are fairly good source of calcium, phosphorous, sulphur, chlorine, iron,vitamin B etc. The nutritive value of brinjals (composition per 100g of edible portion)

according to National Institute of Nutrition, 2007 is calories 24.0g, moisture content 92.7 %, carbohydrate 4.0%, protein 1.4g , fat 0.3g, fiber 1.3g, oxalic acid 18mg, calcium 18mg, magnesium 15mg, phosphorous 47mg, Iron 0.38mg, zinc 0.22 mg, sodium 3 mg, copper 0.12mg, potassium 2 mg, sulphur 44mg, chlorine 52mg, vitamin 124 I.U., folic acid 34μg, thiamine 0.04 mg, riboflavin 0.11mg, β-carotene 0.7 μg ,vitamin C 12mg and amino acids 0.22mg.

The egg plant is also widely used in medicine. According to Ayurvedic system, the white varieties are said to be good for patients suffering from diabetics. Root of eggplant is having anti-asthmatic properties. Leaves are said to possess narcotic properties, and are used in cholera, bronchitis, dysuria and asthma. Its juice is diuretic and used to cure otitis and toothache. The eggplant is reported to promote metabolism of cholesterol. Both leaf and fruit either fresh or dry produces a marked drop in blood cholesterol level. The use of eggplant against cancer disease has also been reported (Hartwell, 1971).

Infact, the vegetable production in every part of the world is impaired to a greater or lesser extent by nematode pests. In more recent years, the nematode diseases of vegetables have increased greatly reducing its economic importance that may sometimes reach to catastrophic proportions. Of all the nematodes found attacking vegetables the root-knot nematode, *Meloidogyne* spp. is considered to be the most important. Collectively, the species of root-knot nematodes are considered among the top five of the major plant pathogens and one or more species attack nearly every crop responsible for the world's supply of food (Sasser, 1979), fiber, timber, resins, ornamentals or other cash crops.

All the species of the genus *Meloidogyne,* causing root-knot disease, stand out as the most dominant group of plant parasitic nematodes in almost every vegetable field and cause enormous losses every year, both in the nursery and planted fields. Nearly all vegetables have been reported as hosts for root-knot nematodes and same vegetable may be invaded by more than one species

of this nematode. Root knot nematodes are obligatory sedentary endoparasites with wide host range that encompasses more than 2000-3000 plant species (Abad *et al.*, 2003, Agrios, 2005). More than hundred species of *Meloidogyne* (Eisenback and Triantaphyllou, 1991) have been described and only four have been recognized as the major and widely distributed species attacking vegetables (Eisenback *et al.*, 1981). These occur in the following order; *Meloidogyne incognita* (Kofoid and White) Chitwood, 47%; *Meloidogyne javanica* (Treub) Chitwood, 40%; *Meloidogyne arenaria* (Neal) Chitwood, 7% and *Meloidogyne hapla* Chitwood, 6% (Sasser, 1980).

So-far eleven species of root-knot nematode *(Meloidogyne)* have been reported from India. Of these, *Meloidogyne incognita* and *Meloidogyne javanica* have the widest host range covering over 232 and 144 genera of plants in India, respectively (Krishnappa, 1985). Many more hosts have been recorded thereafter. Both *M. incognita* and *M. javanica* infestation is common in brinjal. Of the two, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood (1949) is widespread, destructive and the most difficult pathogen of the brinjal, occurring especially in tropical and subtropical climates. It reduces tremendously both quality and quantity of brinjal fruits. The nematode infects the roots and cause root galls. The affected plant becomes stunted and the leaves show chlorotic symptoms. Gaur (1973) founded yield reductions of 17-81 per cent in egg plant depending upon the level of inoculum of *Meloidogyne incognita.* The losses are further increased when pathogenic fungi, bacteria, virus, mycoplasma, other nematodes or insects join the root-knot nematode.

Plant-parasitic nematodes (PPN's) account for worldwide losses of between 5% and 12% annually in various crops (Barker and Koenning, 1998), with root-knot nematodes, *Meloidogyne* spp. being a major cause of such losses (Sasser and Freckman, 1987). Tropical and subtropical climates provide ideal conditions for PPN populations and consequently, the damage caused by them. Managements of PPN's in such climates is a challenge because few control

measures are effective (Schmitt and Sipes, 1998). Chemical nematicides can be effective, but they are often highly toxic synthetic pesticides and are available only to commercial growers. These products are restricted for use on particular crops on a large scale and moreover, it is required usually to be purchased by licensed pesticide applicator.

However, sole reliance on chemical nematicides is not sustainable because of the associated problems of environmental degradation, development of resistance by pest, pest resurgences and secondary pest outbreaks due to elimination of natural enemies. Due to these reasons and the limited availability of nematicide and high cost of nematicides have created a need to discover alternative methods of plant parasitic nematode managements. Availability of biocontrol agents is quite meager in the commercial market in spite of its efficacy in the control of nematodes and the least hazardous effects on environment.

Various biocontrol agents like fungi, bacteria and viruses have been used to control nematodes and another important group which is emerging as potent biocontrol agent of pests are of entomopathogenic nematodes (EPN's).

Nematodes associated with insects are commonly referred to as entomophilic, entomogenous or entomopathogenic nematodes (EPN's). The most commonly found insect parasitic nematodes species belongs to the families Allantonematidae, Mermithidae, Steinernematidae (Chitwood and Chitwood, 1937) and Heterorhabditidae (Poinar, 1976). Many types of association exists between nematodes and insects in the above families, ranging from phoretic (nematodes merely use the insect for transportation) to parasitism (with effects on the insect varying from none to severe/death) and pathogenesis. The families Steinernematidae and Heterorhabditidae, which occur in the latter category, are unique because:-

(1) They are the only nematodes which have evolved the ability to carry and introduce symbiotic bacteria into the body cavity of insects

(2) they are the only insect pathogens with a host range which includes the majority of insect orders like Coleoptera, Lepidoptera, Diptera, Orthoptera, Homoptera etc. and families, and

(3) They can be cultured on a large scale on/or in artificial solid or liquid media.

Steinernematid and Herterorhabditid nematodes have other significant attributes. They can kill insects within 48 hour, can form a durable, infective stage which can be stored for long periods and applied by conventional methods, and persist in the natural environment. EPN's are adaptable biological organisms, natural populations of pests would not be expected to acquire immunity against them. In addition, plants and mammals are not adversely affected. Therefore, these pathogens are attractive from biological and commercial perspective. These qualities have led to a strong commercial interest in the development of these nematodes as biocontrol agents. Several companies are producing and selling nematodes in Europe, U.S., Canada and Australia. Formulations are on clay, alginate gel, polyacrylamide or flowable gel. Bedding (1981, 1984) developed a cheap method for culture of nematodes on offal on crumbled polyurethane sponge.

Currently, EPN's account for most of the biopesticides marketed in the industrialized countries than all the other organisms combined, apart from *Bacillus thuringiensis* (Bedding, 1996). While in many developed countries EPN's are at commercial production stage with identified markets, India lags far behind. A Mermithid, *Romanomermis culicivorax* was briefly sold commercially in the 1970's. However, it was commercially unsuccessful because of environmental limitations (Petersen, 1984). In contrast, nematode species within the families Steinernematidae and Heterorhabditidae are commercially more successful. Currently, *Steinernema carpocapsae* (Weiser) *S.*

feltiae (Filipjev), *S. glaseri,* (Steiner), *S. riobravies* (Cabanillas, Poinar, Raulston), *S. scapteriscae, Steinernema sp., Heterorhabditis bacteriophora* (Poinar 1976) and *H. megidis* are available commercially in different countries (Hom, 1994). Private industries, National Agricultural Research Systems(NARS),Non-Governmental Organizations(NGO's) and International Agricultural Research Centers (IARC) should come forward and become involved in producing EPN products and also popularizing them among farmers. Currently, several private companies have started production and marketing of EPN's for nematode control in India. For example, Ecomax Agro Systems is marketing two EPN's with trade names Soil Commandos (*Heterorhabditis bacteriophora*) and Green Commandos (*Steinernema carpocapsae*) in India. Soil Commandos is recommended for root pests and pathogens and Green Commandos for foliar pests and pathogens. However, greater research and development and extension efforts are required to identify and popularize the EPN–based products for nematode control. A synergistic collaborative programme involving the IARC, NGO's and NARS is needed to foster this environmentally friendly pest & pathogen management approach.

EPN's like other nematodes are thread like, colourless, thin, unsegmented, bisexual, bilaterally symmetrical and eel-like worms. At present, the family Steinernematidae (Chitwood and Chitwood, 1937) is represented by two genera *Steinernema* (Syn. *Neoaplectana*) Travassos, 1927, *Neosteinernema* Nguyen and Smart, 1994. The family Heterorhabditidae (Poinar, 1976) contains a single genus, *Heterorhabditis* (Poinar, 1976). The main difference between the two families is that Stinernematidae shows a conventional amphimictic life cycle (represented by morphologically distinct females) whereas Heterorhabditidae possess a heterogenic life cycle with hermophroditic and amphimictic females.

For laboratory experiments, nematodes can be mass-produced in susceptible hosts. Although Steinernematid and Heterorhabditid nematodes can

infect and reproduce in a wide variety of insect-hosts, not all are suitable for their mass production. Late instar larvae of *Galleria mellonella* (L.), *Corcyra cephalonica* (Stainton) and *Helicoverpa armigera* are generally suitable for maximum nematode yields.The infective stage, known as the infective juvenile (IJ), which is third stage /acquires bacteria from the environment and becomes infective (IJ's) and retains cells of symbiotic bacteria in the specialized intestinal vesicle (Steinernematidae) or in the 2/3 anterior part of intestine (Heterorhabditidae). When it encounters a suitable insect it enters via mouth, anus or spiracles and penetrates into the haemocoel (Heterorhabditids with the help of tooth can also enter through the cuticle of more fragile insects). In the haemocoel the nematode releases the bacteria, which proliferate and cause septicaemia (blood-poisoning) and death of insect within 24-72 hours. The bacteria provide nutrients for the nematodes and inhibit the growth of many micro-organisms. The nematodes pass through several generations in the insect host and eventually emerge as infective juveniles (at 18-28c^0 usually after 18-20days) carrying the bacteria to the next host. The bacteria also produce stilbene and indole metabolites that are nematicidal to a range of nematode species, including some plant parasitic ones (Hu *et al.*, 1995, 1996, 1999).

EPN's have shown some potential as antagonists to PPN's. Applications of EPN's to soil have reduced a number of important PPN species including *Meliodogyne* spp. (Grewal *et al.* 1997; Ishibashi and Choi, 1991; Ishibashi and Kondo, 1987; Smitley *et al.,* 1992), *Belonolaimus* spp. (Grewal *et al.*, 1997), *Tylenchorhynchus* spp. (Smitley *et al.,*1992), and *Criconematidae* (Grewal *et al.*,1997, Ishibashi and Kondo, 1987). Perry *et al.*, (1998) reported a reduction of *Globodera rostochiensis* penetration in potato tubers treated with *S. carpocapsae* in greenhouse and outdoor trials. EPN's tested in laboratory (Bird and Bird, 1986; Grewal *et al.*, 1999) and green-house (Gouge *et al.*, 1994) studies, and applied to tomato plant inoculated with *Meloidogyne* spp. reduced nematode penetration and egg production. Antagonistic interactions between

EPN and PPN were first shown by Bird and Bird (1986), who showed that a reduction of the infection of *M. javanica* in tomato plant was caused by *Steinernema glaseri* (Steiner) in greenhouse pot tests. Similarly, *S. glaseri* DD-136 and *S. feltiae* (Filipjev) reduced populations of PPN and increased the populations of bacteriophagous Rhabditids nematodes (Ishibashi and Kondo, 1986). Lewis and Grewal (2006) reviewed the literature describing these interactions and have found that, while antagonism exists in many cases, the amount of PPN reduction caused is rarely to a level that would be considered acceptable in most agricultural settings.

In laboratory experiments, Grewal *et al.* (1999) observed that penetration of *M. incognita* in tomato seedlings was suppressed by the application of dead infective juveniles (Ij's) killed by thermal shock, but not with live infective juveniles (Ij's) application. Later, Jagdale *et al.* (2002) observed that application of both dead and live infective juvenile (Ij) of *S. carpocapsae* reduced the population in boxwood. Lewis *et al.* (2001) showed that *S. feltiae* and its symbiotic bacterium, *Xenorhabdus bovienii* (Akhurst), affected the infectivity of *M. incognita* in tomato roots. On the other hand the effect of *S. feltiae* application on second stage juveniles (J_2) and on the penetration of these in roots was not evaluated. Recent studies performed by Shapiro-Ilan *et al.* (2006) showed that the number of *M. partityla* (Kleynhans) egg masses was lower compared to control when *S. riobrave* Cabanillas, Poinar and Raulston (7-12) was applied in walnut seedlings. Perez and Lewis (2004) evaluated the effect of the EPN *S. feltiae, S. riobrave* and *H. bacteriophora* Poinar against *Meloidogyne* spp. infecting tomato and peanut seedlings and found that *S. feltiae* and *S. riobrave* reduced *M. hapla* penetration into roots. However, the application of EPN's does not always reduce PPN populations, and the outcomes of their interaction vary according to EPN species, PPN species, the crop receiving the application and the method used to evaluate the impact on the PPN (Lewis and Grewal, 2006).

A number of interactive effects may be involved in suppression of PPN's by EPN's. Bird and Bird (1986) proposed that spatial competition at the mutually attractive root tips may affect root-knot nematode penetration. Ishibashi and Kondo (1986) suggested increased numbers of predators from the application of additional nematode biomass. Grewal *et al.* (1999) found no suppression of PPN's by living EPN's, but did find that applications of dead *S. feltiae* and *S. riobrave* temporarily suppressed root penetration by *M. incognita*. They argued that allelo-chemicals released after the death of the nematode affected root penetration by *M. incognita*.

In view of the above mentioned alternate management method, the present study, " Studies on entomopathogenic nematodes and their role as bio-agents for the management of root knot nematode (*Meloidogyne incognita*) infecting Brinjal" was been selected and the aim was to isolate, mass multiply, develop formulations of EPN's and to apply them on root-knot infected brinjal plants in the pot experiments to assess their effect on the management of *M. incognita* either alone or in combination with fungal bio-control agents(*Paecilomyces lilacinus* and *Trichoderma harzianum*), oil-cakes(neem and soyabean cakes)and nematicide-carbofuran. The experiments carried out are mentioned as follows:-

1. Studies on the frequency of occurrence of entomopathogenic nematodes in Aligarh district of U.P. (India).
2. Studies on the frequency of occurrence of entomopathogenic nematodes in two districts of Jammu and Kashmir (India).
   - **(i)** Pulwama District
   - **(ii)**Shopian District
3. Effect of application of entomopathogenic nematodes (EPN's) at different concentrations on the growth of eggplants.

4. Management of *Meloidogyne incognita* infecting eggplant by using *Steinernema* sp. during concomitant inoculation.
5. Management of *M. incognita* infecting eggplant by the sequential inoculation of *Steinernema* sp., one weak prior to *M. incognita*.
6. Management of *M. incognita* infecting eggplant by the sequential inoculation of *Steinernema* sp., one week after *M. incognita*.
7. Effect of bio-control agents, oil cakes and nematicide-carbofuran on the growth of eggplant.
8. Management of *M. incognita* infecting eggplant by using bio-control agents, oil-cakes and carbofuran alone or in combination with *Steinernema* sp.

Chapter 2

REVIEW OF LITERATURE

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REVIEW OF LITERATURE

Nematode parasites of insects have been known since the 17th century, but it was only in the 1930's that serious consideration was given for using a nematode as a means to control an insect. In 1929, Glaser and Fox found a nematode infecting grubs of the Japanese beetle, *Popillia japonica,* at the Tavistock Golf course near Haddonfield, New Jersey. Steiner described the nematode in the same year as *Neoplectana* (= *Steinernema) glaseri.*

Glaser was the first to cultivate an entomopathogenic species on solid media axenically and the first to conduct the field experiments with cultured nematodes against an insect pest, the Japanese beetle.

Steinernematids and Heterohabdits have been artificially cultured on a variety of substrates viz., veal infusion agar (Glaser 1932), Potato mass (McCoy and Glaser, 1936), ground veal pulp (McCoy and Girth, 1938).

Steiner (1923) described the first nematode in this group as *Aplectana kraussei.* Travossos (1927) erected a new genus for the species as *Steinernema kraussei* but there has been inclusion of two different species in this group (*feltiae* and *carpocapsae*) according to Poinar, 1990 and *feltiae* and *kraussei* according to Gwynn, 1993.

Ahmad and Leather (1994) reported that the annual increase in insecticides is one to two percent and that of microbial insecticides 10 to 25 percent. Nematodes associated with insects referred as entomophilic nematodes are known to parasitise and kill the insects. The entomopathogenic nematodes are the potential agents as they serve as vectors of bacteria and achieve a quick mortality of the target insect pest.

Significant developments have been continued over the last 70-80 years. The relationship between nematodes and their symbiotic bacteria has been revealed and explored since long back. Additional nematode species have been discovered and studied. Finally, field experiments have shown that these

nematodes have potential to be used as biological insecticides against a range of soil and other insects. Unfortunately, much of the information concerning production and application of these nematodes is currently found in patents and confidential reports of commercial companies, and is unavailable to the public.

2.1. Survey, collection & Isolation of different populations of EPN's:-

EPN's have been recovered from many regions throughout the world (Woodring and Kaya, 1988), except Antarctica (Griffin *et al*,1990). Steinernematids were observed to be more abundant in temperate climates whereas Heterorhabditids were abundant in tropical climates.

Beavers *et al* (1998) carried out a monthly survey of the subterranean natural enemies of *Diappeps abbrevitus* (L.), from June, 1979 to December, 1980 in nine citrus groves and one ornamental nursery in central Florida, U.S.A. They found EPN'S *Steinernema feltiae* (*Neoplectana carpocapsae*) and *Heterorhabditis* species active in soil and infecting *D. abbreviatus* larvae throughout the year.

Akhurst and Bedding (1986) recovered four species of *Heterorhabditis* and nine species of *Steinernema* isolated from Tasmania, Australia.

Poinar *et al.* (1987) reported a new species of EPN *Heterorhabditis megidis* from the Ohio University.

Mamiya (1988) isolated *Steinernema kushidai* from soil samples of Central Japan. This is the first report of an EPN's from Japan. This nematode is successfully used for the control of larvae of several species of scarbaeid bettles in Japan.

Hominick and Briscoe (1990) studied the presence of EPN's in soil from 15 sites in Berkshire and survey U.K., making 10 samplings over a period of 28 months. Most of the nematodes obtained were identified as *Neoplectana bibionis*. Soil from verges or pasture usually resulted in more infected larvae, more frequently than soil from other habitat types.

Griffin *et al.* (1991) surveyed insect parasitic nematodes in the Republic of Ireland, between October, 1986 and October, 1987. A total of 551 soil samples were tested for the presence of EPN's with *Galleria mellonella* baiting technique. *Steinernema feltiae* and *Steinernema affinis* were recovered from 7.1 and 3.3% of samples respectively while *Heterorhabditis* species was found only in one sample.

Singh *et al.* (1992) conducted survey at ICRISAT centre between June and December 1991 for the presence of *Steinernema* and *Heterorhabditis* nematodes. Soil samples were collected from 110 sites in 5 vertisol and 6 alfisol fields and nematodes were baited with larvae of *Corcyra cephalonica.* Populations of *Steinernema* species (probably *Steinernema feltiae)* were found only in soils collected from 2 vertisol fields. Morphometric data of infective nematode juveniles collected from the soil were listed.

Amarsinghe *et al.* (1994) described two species of *Steinernema* from Srilankan soil.

Elawad *et al.* (1997) isolated the *Steinernema abbasi* sp. from soils in alfa-alfa in sultanate of Oman. This nematode was also isolated from sub-tropical and semi-arid environment where the boll worms (*Helicoverpa armigera*) and *Spodoptera litoralis* are major pests. They used morphological characteristis, DNA analysis and hybridization technique to show that *S. abbasi* is distinct from *S. carpocapsae, S. scapterisci* and *S. riobravae.*

Gulsar Banu *et al.* (1998) observed the natural occurrence and isolated an EPN, *Heterorhabditis indica* (Poinar *et al.*) from the soil. It was reported for the first time from Kerala, India.

Rajkumar *et al.* (2001) collected 105 soil samples from cultivated and uncultivated fields in and around Udaipur district of Rajasthan. Nematodes were isolated by baiting technique. Out of 105 samples only 5 samples yielded EPN's belonging to the genus *Steinernema* and *Heterorhabditis.*

Hussaini *et al.* (2001) recovered three species of *Steinernema*, namely, *S. tami*, *S .abbasi*, and an undescribed *Steinernema* sp. SSL2 from the soil samples by using *Galleria* larvae.

Ganguly and Singh (2001) collected 105 soil samples from the rhizosphere of various fruit trees, ornamental crops, field crops, weeds and grasses from the IARI form area of New Delhi. The soil samples were baited with last instar larvae of greater wax moth (*G. mellonella*). The EPN's belonging to the genera *Steinernema* and *Heterorhabditis* were recovered only in 15% soil samples, from the rhizosphere of lemon, cotton and mungbean.

Parihar *et al.* (2003) collected 477 soil samples from the rhizosphere of various crops in 14 districts of Rajasthan for the occurrence of EPN's during September 2001 to August 2002. Out of these, 3 samples from rose and 4 samples from maize in Udaipur district yielded *Steinernema* sp. and *Heterorhabditis* sp., while out of 27 samples collected from Jaipur district, only one sample from mango showed the presence of *Heterorhabditis* species.

Gulsar Banu *et al.* (2005) surveyed for the presence of naturally occurring EPN's throughout the 14 districts of Kerala state. *Heterorhabditis* were found to occur more (6.7%) than *Steinernematids* (2.5%). EPN's recovered were identified as *Heterorhabditis indica* and *Steinernema siamkayii*. They are widely distributed in areas receiving annual rainfall of 1500-2000 to more than 3000mm. EPN's were found to occur more in sandy loam soil (13.8%) than in sandy soil (10%).

Siddiqui *et al.* (2005) collected 97 soil samples from the rhizosphere of various crops grown in varied agro-climatic zones of Rajasthan during Rabi. Out of these, only six samples collected from wheat, rose, guava and forest trees yielded EPN's belonging to genera *Steinernema* and *Heterorhabditis*. They were designated as race STSIRO of *Steinernema* and HMABU of *Heterorhabditis*.

2.2. Occurrence of EPN's in varied Agro-climatic zones:-

Hsiao *et al.* (1998) studied the effect of cropping practices on populations of the EPN, *Steinernema carpocapsae,* in three crops in Taiwan; populations of *S. carpocapsae* in fallow and bare plots were very low in February and March 1988, due to snowfall. Higher populations of *S. carpocapsae* were found in non-tillage and conventional tillage maize, sorghum and soybean fields decreased overtime from October 1988 to April 1989. Five months after application, no infective juveniles were recorded from most of the plots, except for those which had received a conventional tillage maize treatment. The number of *S. carpocapsae* in soil covered with maize and sorghum debris was significantly lower after 30 days at 25^0C. In the horizontal movement study, *S. carpocapsae* was capable of moving 3.5 cm per day in bare soil plots and 7.5cm in rye mulch-covered plots indicating that the mulch enhanced movement in agriculture systems.

Ganguly and Singh (2000) reported a new species of EPN, *Steinernema thermophilum* from farm area of the IARI, New Delhi. This is the first record of a new species of *Steinernema* from India.

Sosama and Rasmi (2001) collected 430 soil samples from seven districts of Kerala covering different soil types. Nematodes were isolated by baiting technique using *Corcyra cephalonica* (Stainton) larvae maintained on white traps. *Heterorhabditis indicus* occurred in 128 samples and *Steinernema* sp. in one sample. However, largest percentage occurred in sandy loam soil.

2.3. Host Range of EPN's:-

Chaudhari *et al.* (1995) observed that a Mermithid nematode was found infecting the fifth instar larvae of *Antheraea mylitta* (ist generation) in the fields in India. Mortality of the larvae averaged 1.50 percent in 1990, 2.20 percent in 1991 and 1.70 percent in 1992.

Smith (1996) observed that EPN's have been successfully commercialized as biological control agents for a variety of curculionid species and these are reviewed here. Nematode based products have been introduced

for root weevils, *Otiorhynchus* sp., billbugs, *Sphenophorus* sp., the apopka weevil, *Abbreviatus* and citrus root weevil, *Pachnaeus litus*. Several species of other *Curculionidae* species and some other related families are potential candidates for future commercial introductions.

Mathur *et al* (1996) concluded that laboratory assessment of the virulence of *Steinernema carpocapsae* to cockroach, *Periplaneta americana* using filter paper exposure method at 25^0C revealed that 5,000 infective juveniles per insect caused 70 percent and 50 percent mortality in males and females respectively.

Vela *et al.* (1998) evaluated the effectiveness of the genus *Steinernema* on *Helicoverpa armigera* in the laboratory. In addition, techniques for the laboratory rearing of *H. armigera* are described. The results showed higher susceptibility of the larva (100 percent mortality) for this EPN.

Karunakar *et al.* (1999) concluded that among the nine lepidopterean laboratory hosts screened, *G. mellonella, C. cephalonica H. armigera, Spodoptera litura* and *Schirphaga excerptalis* were highly susceptible.

Kaushal *et al* (2000) showed that four populations, two of *Heterorhabditis* from Ghaziabad (19 GAZ) and Aligarh (1 AMU) and two of *Steinernematids* from Ghaziabad (3 GAZ) and Himachal Pradesh (31 HP) were used. Among these four, 1 AMU, and 31 HP were the most virulent. *Periplaneta americana, Acheta domesticus* and *Odontotermes obesi* were tested for differential susceptibility to infection.

Riga *et al.* (2001) performed a series of experiments in the laboratory and in the green house to assess the efficacy of EPN's viz., *S. glaseri* or *S. feltiae* decreased the numbers of European maize borer, fall army worm, western maize root worm and the seed corn maggot in maize. Both nematode species effectively controlled the four insect pest species.

Razak and Sivakumar (2001) tested the host range of the EPN *S. feltiae* in the laboratory using 17 insect species. *S. feltiae* infected 13 sp., while three *sp., Holotrichia serrata, Nilaparvata lugens* and *Aphis craccivora* and one natural

enemy, *Chilomenes sexmaculata,* could not act as hosts i.e., *S .feltiae* could not reproduce in them. However, ij's of *S. feltiae* infected *Chilomenes sexmaculata, Nilaparvata lugens* and *Aphis craccivora,* and caused mortality within 2 days of inoculation.

2.4. Mass multiplication of EPN's:-

2.4.1. *In-vivo* mass multiplication:-

Karunakar *et al.* (1992) studied the production of *Steinernema glaseri* and *Heterorhabditis indica* on sugarcane internode borer *Chilo sacchariphagus indicus* (Kapur). They found that, on an average, 37,335.8 ij's/larvae in *S. glaseri* and 210,283.3 ij's/larvae in *H. indica,* when both were inoculated at the rate of 25 ij's/larvae. Further, they concluded that the multiplication ij's/unit body weight of the host was higher in *Heterorhabditis indica,* than *S. glaseri.*

Hussaini *et al.* (1998) reported the nutritional requirement of infective juveniles of native EPN's, *Steinernema* species from insect hosts, *Galleria mellonella, Agrotis ipsilon, Spodoptera litura, H. armigera* and *C. cephalonica. G. mellonella* were found to be the most suitable host for *in vivo* production than other insects.

Zaki *et al.* (2000) studied *in Vitro* culturing of EPNs, *H. bacteriophora* and *S. carpocapsae* on silkworm, *Bombyx mori (L.).* Both *H. bacteriophora and S. carpocapsae (500* nematodes/larvae*)* caused 100% mortality of the fifth instar larvae of *Bombyx mori* after 24hr and 48 hr of application in case of injection and topical methods, respectively. The average number of nematodes that emerged from the third instar larvae of silkworm was 2750.

Rajkumar *et al.* (2002) studied mass multiplication of *Steinernema* species and *Heterorhabditis* species on greater wax moth, *G. mellonella.* The ij's of *Steinernema* species and *Heterorhabditis* species were inoculated on three categories of *G. mellonella* based on the larval size and body weight. The difference was recorded in both the species with regard to the size and body weight of *G. mellonella* larvae.

Gaugler *et al.* (2002) reported one component of nematode production in the U.S.A in a cottage industry of low volume producers using *in vivo* technology, based on a method devised in 1927, the white trap. They reported the first scalable system for *in vivo* nematode mass production. Unlike the white trap, there is no requirement for nematode migration to a water reservoir. The LOTEK system of tools and procedures provides process technology for low cost and high efficiency mass production. The harvester collects 97% of *H. bacteriophora* (Poinar) that emerged from *G. mellonella* (L.) cadavers in 48 hours. The Separator removes 97.5% of the wastewater in three phases, while nematode concentration was increased 81fold.

2.4.2. *In-Vitro* mass production:-

Kaiji *et al.* (1998) studied the types of metabolites produced by *P. luminescens* C9 when it was introduced by *H. megidis* 90 into *G. mellonella* larvae are different from those produced in tryptic soya broth. Only 3,5-dihydroxy-4-isopropyl stilbene 1 was identified from the organic extracts of *P. luminescens* culture broth, but both 3,5-dihydroxy 4-isopropyl stilbene 1 and 3,5-dihydroxy-4-ethyl stilbene 3 were isolated from the organic extracts of nematode bacterium infected *G. mellonella* larvae. In addition to two pigments, both of which have been previously reported from *P. luminescens* C9 culture broth, 3 pigments, 1,8-dihydroxy 3-methoxy-9, 10-anthraquinone 2, 1-hydroxy 2, 6, 8-trimethoxy-9, 10-anthraquinone and 1, 4-dihydroxy-2, 5-dimethoxy-9, 10-anthraquinone 7 were isolated from the organic extracts of *G. mellonella* larvae infected by the nematode-bacterium complex. Among these compounds 6 and 7 were novel and were isolated from a natural source for the first time.

Hussaini *et al* (2000) carried out mass multiplication of *Steinernema* sp. (SSL2) PDBC EN 13.21 in four combinations of dog biscuit media in comparison with wouts medium. The cost of production was evaluated. After a culture time of 30 days and an initial inoculum of 500 ij's per 250 ml a maximum yield of 30.58×10^5 ij's was recorded from wouts medium followed by dog biscuit + peptone + beef extract (24.5×10^5), dog biscuit + beef extract

(18.40×10^5), dog biscuit + peptone (12.20×10^5) and dog biscuit + bacterial culture (10.14×10^5). The cost of production for 10 lakh ij's was highest for dog biscuit + bacterial culture followed by dog biscuit + peptone + beef extract, dog biscuit + peptone, dog biscuit + beef extract and wouts medium: Wouts medium was found to be the best both in terms of yield/gram of medium and cost of production per 10 lakh ij's.

Vyas *et al.* (2001) attempted *in vitro* mass production of native *Steinernema* species using 21 animal and plant protein based media. Maximum production of nematodes was recorded in hen egg yolk medium which was economically better than the universally used dog food biscuit agar. Production of EPN's was poor in plant protein compared to animal protein-based media.

Gulsar Banu and Rajendran (2001) have tried different media, wouts medium, wouts medium supplemented with Bengal gram flour or green gram flour instead of soya flour and coconut oil or groundnut oil instead of corn oil, under laboratory conditions at 25^0C. Medium was coated on 1 cm^3 polyurethane foam and *Steinernema* species isolated from Kerala was inoculated at the rate of 400ij's/flask. Maximum multiplication was recorded in wouts medium.

Han *et al.* (2001) reported *Photorhabdus luminescens* is a symbiont of EPN, *Heterorhabditis* species used for biological control for insect pests. For industrial mass production, the nematodes were produced in liquid media, pre-incubated with their bacterial symbiont, which provides nutrients essential for the nematode's development and reproduction.

Johnigk *et al.* (2002) observed that the ij of EPN's (*Heterorhabditis* species) is the mobile, but developmentally arrested dauer juvenile (Dj). For commercial application, nematodes are produced in liquid culture; prior to inoculation of the Dj, their symbiotic stage (recovery) and develop to reproductive adults. In liquid culture the percentage of Dj's recovering from the Dj stage is highly variable, which significantly influenced the number of reproducing hermaphrodites and the final Dj yields. A hybrid strain of *H.*

bacteriophora- 30 homozygous inbred line was propagated in liquid culture and Dj recovery and yield were recorded. The calculated heritability for the Dj recovery was low (h^2 = 0.38). No significant genetic variability could be detected for this trait. In contrast, a high heritability (h^2 = 0.90) was found for the total number of Dj's produced in the liquid medium.

2.5. EPN-based Formulations:-

EPN's are used as drenching or injecting into soil (Georgis & Hague, 1982), drip irrigation (Reed *et al.* 1986), encapsulating in the calcium alginate (Kaya and Nelsen 1985) and nematode embedded hydrogels (Poinar *et al*, 1985).

Grewal *et al.* (1995) reported that the new formulation holds large numbers of practically dessicated nematodes in small granules, nematode energy burn-rate was significantly reduced, extending the shelf life of *Steinernema carpocapsae* to 5-6 months at room temperatures.

Shapiro-Ilan *et al.* (2001) determined that the feasibility of formulating nematode infected insect cadavers to overcome these hindrances. All experiments were conducted with *H. bacteriophora* and *G. mellonella*. Non formulated cadavers were used as controls. A total of 19 formulations were tested (including combinations of starches, flours, clays, etc.). Starch-clay combination was found to adhere to the cadaver and had no significant deleterious effects on nematode reproduction and infectivity; other formulations exhibited poor adhesion or reduced nematode reproduction. Formulated cadavers were more resistant to rupturing and sticking together during agitation than non formulated cadavers.

Narkhedkar *et al.* (2002) developed the formulations of EPN's for the biological control of *H. armigera*. Eight materials (animals fat, guar gum, glycerol, aloe gel, sunflower oil, carboxy methyl cellulose (CMC), *Acacia* gum and agar-agar), which have anti-evaporant properties, were tested against five isolates of *Heterorhabditis indica* under laboratory conditions. The nematodes were tested for their infectivity before and after the treatment. Guar gum,

glycerol, aloe gel, sunflower oil and CMC at 10%were effective, compared to control. Viability was found best with suspension while for infectivity, both suspension and slurry were at par.

Lewis and Perez (2004) revealed that the type of formulation of EPN-based products influences their handling, storage and application requirements. Different EPN Species can have different temperature, moisture and oxygen requirements. These requirements can dictate in many cases how nematodes were formulated and stored. The combination of EPN species and formulation types can result in a vast array of products. They are used for management of many different insect pests in several different commodities. EPN's are living organisms and regardless of how they are formulated, their activity declines with time. Furthermore, all formulations are susceptible to temperature extremes, ultraviolet light, anoxic conditions, and contamination. The function of a formulation is to increase nematode shelf life and to facilitate storage and application.

Wilson and Ivanova (2004) developed a non-viscous, non-adhesive and non-toxic liquid formulation for nematode storage and transport based on neutral density colloidal silica suspension. Survival and virulence of nematodes stored in this formulation with out aeration was superior to nematodes stored in aerated quarter strength rinser's solution.

Schroer *et al.* (2005) observed that the polymers Arabic and guar gum, alginate and xanthan were used in concentrations between 0.05 and 0.3% to retard sedimentation of *Steinernema carpocapsae*. Arabic gum had no effect, guar gum prevented sedimentation at 0.3% but the effect dropped significantly at lower concentration. At 0.05%, xanthan prevented nematode sedimentation better than alginate. Deposition of nematodes on the leaves was significantly increased by the addition of any of the polymers. Spraying nematodes on leaves with an inclination of 45^0 without the addition of any formulation resulted in 70% run-off. Adding 0.2% alginate or xanthan reduced the losses to < 20%. The use of a surfactant-polymer formulation significantly reduced

defoliation by DBM larvae. Visual examinations provided evidence that DBM larvae did not ingest nematodes. Invasion of *S. carpocapsae* was an active process via the anus. The function of the formulation was not to prolong nematode survival, but to provide environmental conditions, which enable rapid invasion of the nematodes. Nematode performance was improved by selection of the best surfactant in combination with xanthan and by optimization of the concentrations of the surfactant rimulgan and the polymer xanthan. The best control results were achieved with rimulgan at 0.3% together with 0.3% xanthan, causing DBM mortality of > 90% at 80% relative humidity and > 70% at 60%.

2.6. Bioassay of EPN's:-

Forschler and Gardner (1991) recorded the LC 50 values of *Steinernema carpocapsae*, *S. glaseri* and *Heterorhabditis heliothidis* to the third instar *Phyllophaga girfcula* to be 210, 86 and 12 nematodes per grub, respectively. However, due to migration, actual concentration of nematode remained less than actually applied.

Baskaran *et al.* (1994) evaluated the efficacy of *Steinernama carpocapsae* (strain DD-136), *Heterorhabditis bacteriophora* (Strain Burlier) and *Heterorhabditis* sp. (Strain Chekkanurnai and Melur) against fourth instar larvae of *Amsacta albistriga* on groundnut in field trials in Tamil Nadu, India, in 1992. *Steinernema carpocapsae* was the most effective followed by *Heterorhabditis* sp. The host insect died because of the symbiotic bacteria of the nematode *Steinernema carpocapsae* developed in the larva.

Josephrajkumar and Sivakumar (1997) conducted an experiment and found that a native *Steinernema* sp. could be used as soil drench at the dosage of 13×10^4 ij's/m^2 against pupae of *Spodoptera litura* but it was comparatively less virulent than the exotic *Steinernema glaseri.* A greenhouse study showed that the native *Steinernema* sp. and *S. glaseri* were equally effective against *Spodoptera litura* fourth instar larvae when sprayed on black gram at the dosage of 1500 ij's/plant. The nematode infected insect larvae caused

significantly less damage to the leaflets of black gram 48, 72 and 96 hours after spraying.

Hussaini *et al* (2000) investigated infectivity of four native populations of *Steinernema* sp. and *Heterorhabditis indica*, alone and in combinations, against *Agrotis ipsilon* in sand and sandy loam soil columns and observed that the performance of all nematode populations was better in sandy loam soil than in sand at 5 cm depth. The mortality of *Agrotis ipsilon* enhanced with an increase in time of exposure.

Padmanabham *et al* (2001) observed the effects of *Heterorhabditis indica* PDBC 13.3 against post-embryonic developmental stages of the banana stem weevil (BSW) *Odoiporus longicollis* (Oliver) under laboratory conditions using Petri-dish bio-assay and results found that *H. indica* was pathogenic to grubs and adults of BSW.

Patel and Vyas (2001) studied the biological control of potato cutworm, *Agrotis ipsilon* and white grub by using native EPN's (*Steinernema*) isolates of middle Gujrat villages, Vatrak isolates (VI) and Anand isolate (AI).

Vyas *et al.* (2002) conducted a field experiment in Anand, Gujrat, India in 1999/2000 to evaluate the efficacy of *Heterorhabditis* sp. against *H. armigera* infesting pigeonpea C.V. G.T 100. Treatments were given at 100,000 ij's/m^2. All the treatments significantly reduced the population of *H. armigera* and pod damage percentage and increased the pigeonpea yield.

2.7. Ecological studies of EPN's:-

Shamseldean *et al.* (1999) conducted an experiment on the factors affecting pathogenicity of an Egyptian strain of *Heterorhabditis indica* infecting cotton leafworm, *Spodoptera littoralis. Heterorhabditis indica* reported herein possesses better persistence at high temperature in terms of its reproduction efficiency than other EPN Species.

Karunakar *et al.* (1999) conducted an experiment to study the influence of temperature on infectivity, penetration and multiplication of *S. feltiae, S. glaseri* and *H. indica* and found that nematode infection and penetration of *G.*

mellonella was observed from 12.5 to 32.5^{0}C while multiplication of ij's was observed only from 25 to 32.5^{0}C.

Koppenhoper *et al.* (2000) studied the basic ecological characteristic of the EPN, *S. monticolum*. This infected hosts over a wide range of soil moisture ranging from 1500 Kpa to 3 Kpa. It had a wide thermal activity range with optimum infectivity from 12 to 25^{0}C. Because of its foraging strategy and adaptation to cool temperatures, *S. monticolum* has the potential for control of pests, especially Lepidoptera that hibernate in the upper soil layer.

Grewal (2000) has explored the effects of anhydrobiosis on longevity and infectivity of three species of EPN's, *S. carpocapsae, S. feltiae,* and *S. riobrave* at 5 and 25^{0}C. Anhydrobiosis was induced in water-dispersable granules (WG) at 0.966-0.971 water activity and 25^{0}C following a 7 day preconditioning of ij's at 5^{0}C in tap water. Survival and infectivity of desiccated (anhydrobiotic) ij's was compared with non-desiccated ij's stored in water for different period. Anhydrobiosis increased longevity of *S. carpocapsae* ij's by 3 months and *S. riobrave* by 1 month in WG at 25^{0}C as compared with ij's stored in water. However, desiccation decreased longevity of *S. feltiae* at 25^{0}C and of all three species at 5^{0}C. These results have demonstrated a shelf life of 5 months for *S. carpocapsae* at 25^0 C and 9 months at 5^{0}C in WG with over 90% if survival occurred only for 2 months at 25^{0}Cand 5 months at 5^{0}C in WG. *S. riobrave* had over 90% survival only for 1 month at 25^{0}C and the survival dropped below 85% within 1 month at 5^{0}C induction of anhydrobiosis in WG resulted in 85, 79.

Csontos (2001) conducted an experiment to find out the lateral movement and host location of *Steinernema glaseri* in sand at different temperatures and concluded that the ability of the infective juveniles to find the host was mainly influenced by the temperature as infective juveniles reached their host quicker as the temperature rose.

Hussaini *et al.* (2001) conducted an experiment to see the effect of different temperatures on the infectivity and progeny production of indigenous

EPN isolates. It was recorded that increase of temperature from 15 to 32^0c was favourable for infection and progeny production.

Gavas and Ganguly (2001) studied the effect of soil moisture on infectivity of *Steinernema thermophilum* to know its optimum moisture requirement. Due to its wide range of moisture requirement, *S. thermophilum* may prove to be a useful bioagent in arid and semi-arid, subtropical and tropical climatic conditions prevailing in different regions of world.

Effect of soil depth on infectivity of *S. thermophilum* was studied by Gavas and Ganguly (2002). It showed that *S.thermophilum* could infect the host located at soil surface as well as at a depth of 10 cm.

Hussaini *et al.* (2002) conducted an experiment to record the effect of metal ions on infectivity and progeny production of indigenous isolates of EPN's against *Agrotis ipsilon* and *G. mellonella*. It is inferred that a simple chemical treatment could be useful in enhancing the performance of EPN's.

2.8. Pathogenicity of EPN's:-

Pathogenicity of *Steinernema riobrave* against corn earworm, *Helicoverpa zea,* was tested by Cabanillas and Raulston (1994) who found that the production of nematodes was independent of concentration of infective juveniles and pathogenicity of *S. riobrave* and its symbiotic bacterium *Xenorhabdus* to corn earworm and suggested that it may have a great potential against prepupal and pupal stages of *Helicoverpa zea.*

Karunakar *et al.* (2000) tested the pathogenicity of Steinernematid and Heterorhabditid nematodes to white grubs infesting sugar cane in India and found that the mortality caused by both the species of nematodes decreased with an increase in the age of the white grubs.

Pathogenicity caused by high virulent and low virulent strains of *S. carpocapsae* to *G. mellonella* was studied by Simoes *et al.* (2000). For this, they compared the virulence of two strains of *S. carpocapsae,* a highly virulent strain (Breton) and a low virulent strain (Az 27) and found that both the strains

have similar ability to invade *G. melonella* with median infection times of 3.9 and 3.2 hours, respectively.

2.9. A Review on the Management of Root-knot nematode by Entomopathogenic nematodes:

Bird and Bird (1986) investigated those infective larvae of entomogenous nematode, *Steinernema glaseri* orient towards the root tips of germinating tomato seedlings on 0.75% aqueous agar. This behaviour parallels that of the infective larvae of the root-knot nematode *Meloidogyne javanica* under similar conditions in that these larvae also accumulate in the meristematic region of the root. In both instances it seems that carbon dioxide is largely responsible for this orientation. Pot experiments showed that *S. glaseri*, when applied at high concentration (5×10^6 per plant) at one application or at a lower application (5×10^5) daily over 10 days, significantly reduced the numbers and reproductive capacity of *M. javanica* applied at a concentration of 2×10^3 per plant. This effect was approximately 4-fold when expressed as a total egg mass count and approximately 6-fold when expressed as egg masses per gram of fresh wt. of root. In addition to reducing the infestation of plants by root-knot nematodes the application of larvae of *S. glaseri* led to the production of significantly larger plants.

Ishibashi and Kondo (1987) studied the dynamics of *Steinernema feltiae* DD-136 in soils with different fauna to determine the best method for the control of soil insects and plant-parasitic nematodes. Infective juveniles (J_3) were applied to field plots with and without 1, 3-D (Telone II) fumigation. Recovery of J_3 and changes in native nematode fauna were monitored until the applied J_3 were no longer recovered by Baermann Funnel (BF). Recovery of J_3 by BF or by two-step extraction procedure from steam-sterilized or non-sterilized sandy or silty soil with different fauna was investigated. More DD-136 J_3 were recovered from the 1, 3-D treated soil than from non treated soil, while native nematodes in the treated soil fluctuated more with the addition of DD-136 than those in non-treated soil. The j_3 persisted longer in silty than in

sandy soils. The inundative soil application of DD-136 increased native rhabditids and decreased plant parasitic nematode including root-knot nematode. DD-136 in chemically treated soil not only effectively attacked the invading soil insect pests but also suppressed the recovery of plant nematodes.

Choi *et al.* (1988) studies the efficacy of *Aphelenchus avenae* and *Steinernema feltiae* against soil pests and soil-borne diseases of cucumber. Application of *A. avenae* at > 5×10^4 / 500 ml soil effectively suppressed the pre-emergence damping-off of cucumber seedlings due to *Rhizoctonia solani* strain AG-4, but caused a Leaf-miner-like symptom on the cotyledons. Germination of the cucumber seeds was adversely affected by *A. avenae,* but no detrimental effect on further growth was observed. Plant germinated normally when *A. avenae* was applied together with *S. carpocapsae*. The pathogenicity of *S. carpocapsae* to the *Spodoptera litura* was not affected by mixed application of the nematodes, even at concentrations of A. *avenae* 100 times greater than that of *S. carpocapsae*. Mixed inoculation of *M. incognita* with either *A. avenae* or *S. carpocapsae* resulted in less gall formation of cucumber roots than did *M. incognita* alone. Mixed application of *A. avenae* and *S. carpocapsae* not only suppressed the virulence of *R. solani* and *M. incognita*, but also reduced the detrimental effect induced by *A. avenae* inoculation alone. They suggested that beneficial nematodes should be applied with culture medium as a basal dressing to the soil after chemical treatment for the integrated control of soil insect pests and soil-borne diseases of cucumber.

Kermarrec *et al.* (1991) applied *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* simultaneously with *Meloidogyne incognita* to tomato plants in pots. It was reported that the number of *M. incognita* females on the roots was significantly reduced by *S. carpocapsae* while only the reproduction factor of the root-knot nematode was decreased by *H. bacteriophora.*

Ishibashi and Choi (1991) conducted green house experiments in which massive application of the fungivorous nematode, *Aphelenchus avevae* viz., in

summer at 26-33 C^0 (1 × 10^5 nematodes / 500 cm^3 autoclaved soil) and in autumn at 18-23 C^0 (5 × 10^4 nematodes / 500 cm^3 autoclaved soil), suppressed pre-emergence damping-off of cucumber seedlings due to *Rhizotonia solani* AG-4 by 67% and 87%, respectively. Application of 2 × 10^5 *A. avenae* to sterilized soil infected with *R. solani* caused leafminer-like symptom on the cotyledons, which did not occur in mixed inoculations with the entomopathogenic nematode, *Steinernema carpocapsae.* When 1 × 10^6 *A. avenae* were applied 3 days before inoculation with 100 *Meloidogyne incognita* juveniles, gall numbers on tomato roots were reduced to 50% of controls. Gall number was also suppressed by *S. carpocapsae* (Str. All). Reduction in gall numbers was no greater with mixed application of *A. avenae* and *S. carpocapsae* than with application of single species, even though twice the number of nematodes were added in the former case. These nematodes were positively attracted to tomato root tips.

Ishibashi and Choi (1992) revealed that massive application of *Aphelenchus avenae*, with 2 × 10^5 / 500 ml of autoclaved soil in summer at 26-33 C^0 and 5 × 10^5 nematodes / 500 ml in autumn at 18-23 C^0 effectively suppressed the pre-emergence damping of cucumber seedlings due to *Rhizoctonia solani* AG-by 80% and by 87% respectively. However, application of *A. avenae* more than 2 × 10^5 nematodes caused leafminer like symptoms on the cotyledons in fall season without any detrimental effects on the further plant growth. The simultaneous inoculation of root-knot nematodes *Meloidogyne incognita* and *A. avenae* in 1 : 1,000 ratio reduced the number of galls by 30-40 percent of *M. incognita* alone inoculation. Similarly massive inoculation of *Steinernema feltiae* also reduced the galls of M. *incognita.* Mixed application of *A. avenae* and *S. feltiae* suppressed the virulence of both *R. solani* and *M. incognita,* and removed the leafminer like symptoms by *A. avenae.* Since the beneficial nematodes are highly tolerant to conventional dosage of pesticides, mixed application with chemicals may be recommended to integrated control of soil pests.

Tsai and Yeh (1995) observed that the effect of *Steinernema carpocapsae* on the infection rate of plant-parasitic nematodes vary with the change of host plants. On adzuki bean and tomato seedlings, *S. carpocapsae* reduced the infection of *Pratylenchus coffeae* (Zimmermann) Filipjev & Schuurmans Stekhoven by 85% and 88%, respectively, and that of *Meloidogyne javanica* (Treub) Chitwood by 39% and 78%, respectively. However, on radish seedlings it has no effect on *M. javanica* yet increased the infection of *P. coffeae* by 160%. The ratio of Steinernematids to plant-parasitic nematodes was 100: 1 when applied to sterile sand in the above experiments. *S. carpocapsae* did not affect the infectivity of *P. coffeae* on edible rape seedlings on water agar at the application rate of 50: 1. The mechanism of interaction in the triangle of *S. carpocapsae*, plant-parasitic nematodes, and host plants was suggested to be more than just competition for root surface.

Gouge *et al.* (1997) treated cotton fields with the entomopathogenic nematode, *Steinernema riobravis* and Vydate L for the control of plant parasitic nematodes. Short staple cotton grown near Coolidge, Arizona, was treated at a rate of 1 billion and 2 billion *S. riobravis* nematodes per acre, and 0.5 lb a.i. Vydate L per acre. Untreated cotton received an application of water only. Treatments were applied through a subterranean drip system with 12 inch spaced outlets. Applications were made in the daily irrigation cycle of 0.33 inches of water, normal irrigation cycles followed. Products were uniformly distributed over the treated fields. EPN's persisted throughout the 6 week experimental period at the 1 billion per acre rate. However, nematodes applied at 2 billion per acre rate disappeared rapidly. Populations of various plant parasitic nematode species were monitored subsequent to treatment application. Nematodes were extracted using a standard sugar flotation technique and counted in 1 ml slide samples. Both *Meloidogyne incognita* and *Tylenchorhynchus* spp. population were reduced by *S. riobravis* applied at 1 billion per acre rate. Phytoparasitic nematodes were reduced following application of Vydate L, but control was not sustained beyond one week.

Matsunaga *et al.* (1997) tested six species of entomopathogenic nematodes (*Steinernema* and *Heterorhabditis* spp.) and a fungivorous nematode, *Aphelenchus avenae*, for their effects on root invasion of *Meloidogyne incognita* and *Pratylenchus coffeae*. The beneficial nematodes were applied before, at the same time, or after the inoculation of plant nematodes onto *Agrobacterium*-transformed cucumber hairy roots in a Petridish or onto seedlings in pots. All beneficial nematodes tested were more or less suppressive to root invasion by plant parasitic nematodes. In particular, both *Steinernema carpocapsae* and *A. avenae* reduced the root invasion by 97% on agar plate with 1104 nematodes and 76% in sterilized soil with 1106 dose level. *Steinernema* sp. from Malaysia, *S. anomali, S. glaseri, Heterorhabditis bacteriophora* and *A. avenae* were most effective following simultaneous application with plant parasitic nematodes. These were mostly cruiser-type nematodes, whereas *S. carpocapsae* and *S. kushidai*, typical ambusher-type foragers, gave the best results by pre application. *Pratylenchus coffeae* was generally more susceptible to other nematodes than *M. incognita.* In non autoclaved soil, the effect of beneficial nematodes was not so marked as in autoclaved soil.

Grewal *et al.* (1997) studied the influence of entomopathogenic nematodes, *Steinernema carpocapsae* and *S. riobravis*, on natural populations of plant-parasitic nematodes (PPNs) infesting turfgrass in Georgia and South Carolina. *S. riobravis* applied at 6×10^9 infective juveniles (IJs) / acre provided upto 95-100% control of the root-knot, *Meloidogyne* sp., sting, *Belonolaimus longicaudatus*, and ring nematode, *Criconemella* sp., in Georgia, but *S. carpocapsae* has no effect. *S. riobravis* was as effective as the chemical nematicide, Fenamiphos (Nemacur 10 G) at 4 weeks after treatment and more effective at 8 weeks after treatment. In South Carolina, both *S. riobravis* and *S. carpocapsae* applied at 1×10^9 IJs / acre provided up to 86-100% control of the root-knot, sting and ring nematodes. Application of 6×10^9 IJs / acre increased control by only 4-14% over the 1×10^9 dosage. Possible causes of differences

in efficacy of *S. carpocapsae* at the two sites are discussed. It is concluded that *S. riobravis* may provide effective, predictable and economical control of PPN's in turf grass.

Hu *et al.* (1998) reported that *Xenorhabdus* and *Photorhabdus*, the bacterial symbionts of *Steinernema* spp. and *Heterorhabditis* spp., respectively, grow rapidly in the insect's haemocoel and provide nutrients in the dying and dead insect for nematode development. The integument-enclosed cadaver contains a highly nutritious medium that enables exceptional multiplication of the nematodes. Among, the secondary metabolites of the bacteria in culture are three nematicidal substances: ammonia, 3, 5-dihydroxy-4-isopropylstilbene (ST), and a heterocyclic substance (HD). ST, at 100g / ml in an immersion tests, caused almost 100% mortality of *Aphelenchoides rhyntium, Bursaphelenchus xylophilus,* and *Caenorhabditis elegans* but had no effect on juveniles of *Meloidogyne incognita* and *H. megidis.* HD caused mortality of *B. xylophilus* and *M. incognita* at 250-500g/ml, but only paralysis at lower concentrations. ST was present after 24 hours in nematode-infected *Galleria mellonella* larval cadavers at concentrations as high as 3,000g / g wet larval tissue. However, HD was not detectable in nematode-infected *G. mellonella* larvae.

Riegel *et al.* (1998) studied whether the soil application of *Steinernema riobrave* and *Heterorhabditis bacteriophora* reduce root-knot nematodes on squash. Treatments (10 replicates) included microplots with *S. riobrave* or *H. Bacteriophora* at a rate of 4.5×10^5 nematodes / m^3, 1, 3-dichloropropene at 84 liters / ha, and an untreated control. One week after the treatments were applied three Zucchini squash cv. Marketeer seeds were planted in each microplot. Plant stand, number of fruit, and marketable yield were higher, and root-knot nematode galling indices were lower in fumigated plots when compared to the untreated control and *S. riobrave* and *H. bacteriophora*-amended plots. Mean shoot growth in the fumigated plots was twice that of the untreated and *S. riobrave* and *H. bacteriophora* amended plots. *Steinernema riobrave* and *H.*

bacteriphora were not effective in the management of the root-knot nematodes on squash.

Samaliev *et al.* (2000) conducted two experiments, one in the laboratory and the other in a growth room and evaluated the influence of the bacterial symbiont, *Pseudomonas oryzihabitans* from the EPN's *S. abbasi* on the root-knot nematode, *Meloidogyne javanica.* Second stage juveniles of *Meloidogyne javanica* were disorientated in the presence of bacterial symbiont and showed convulsive movements.

Vyas *et al.* (2003-2004) isolated native *Xenorhabdus* bacteria from *Steinernema* spp. and their exo- and endo-metabolites were tested for management of root-knot nematodes on tomato. All the native *Xenorhabdus* (Xeno) isolates showed biochemical reactions which were characteristic features of this genus. Moreover, native *Xenorhabdus* isolates exhibited a positive reaction for lecithinase and negative reaction for lipase, protease and chitinase. The total exo-proteins (mg/ml) produced by *Xenorhabdus* Sc culture was higher whereas, Xeno SrM and OH exhibited an equal quantity of protein production followed by Xeno SA. Both exo- and endo-metabolites of *Xenorhabdus* spp. showed suppressive effect against *Meloidogyne incognita* and *Meloidogyne javanica* pt. 2 on tomato in pots.

Fallon *et al.* (2004) have tested *Steinernema feltiae* MG-14 and the symbiont *Xenorhabdus bovieni* for their glasshouse efficacy against the *Meloidogyne javanica* on several vegetable plants. *S. feltiae* applied for 3-5 consecutive days at rates of 1000 or 10,000 ij's did not affect *Meloidogyne javanica* root penetration and development in glasshouse pot experiments. Infective juveniles were recovered from the cortical tissue of tomatoes or snowpeas and cowpeas. *Xenorhabdus bovienii* applied at 1010 colony forming units (C.F.U.) ml^{-1} reduced root knot nematode penetration in cowpeas but was ineffective in tomatoes or snowpea. *Xenorhabdus bovienii* metabolites had no effect on *Meloidogyne javanica* root penetration and egg production in soybean. Soybean plant growth was unaffected by nematode and bacterial

treatment, biomass was lower in *Meloidogyne javanica* infected soybean, irrespective of treatment, than in non-infected soybean, but the differences between the treatments were non-significant.

Perez and Lewis (2004) studied the effect of EPN's on root-knot nematodes infecting tomato and peanut. In growth chamber experiments, peanut seedlings were inoculated with 300 *Meloidogyne hapla* eggs and 25 infective juveniles/cm^2 of *Steinernema feltiae* or *S. riobrave* or *Heterorhabditis bacteriophora* with the exception of seedling roots treated with *Heterorhabditis bacteriophora, M. hapla* inside roots and egg recovery from seedlings treated with EPN's were significantly lesser than those in the controls. Peanut plants in the greenhouse were infested with 5000 *Meloidogyne hapla* eggs and treated 2 weeks and 1 weeks before, at the same time, 1 week after, or 2 weeks after with 25 or 125 ij's/cm^2 of *S. feltiae, S. riobrave*, or *H. bacteriophora.* Pre and post infection applications of *S. feltiae* suppressed *Meloidogyne hapla* penetration with no egg production. Only pre infestation applications of *S .riobrave* suppressed *Meloidogyne hapla.* Only at higher rate of *Heterorhabditis bacteriophora* infestation of *M. hapla* was suppressed. Tomato plants in the greenhouse were infested with 5000 *M. incognita* eggs and treated with 25 or 125 ij's/cm^2 of *S. glaseri* or *H. megidis* applied at the same time. The low rate of *S. glaseri* suppressed *M. incognita* penetration into tomato roots and the higher rate of *S. glaseri* reduced egg production.

Chaubey *et al.* (2004) revealed that the EPN's were not only harmful to insect pests but also harmful for plant parasitic nematodes (*Meloidogyne* spp.). These nematodes can be applied very easily in the field to control the insect pests as well as the root knot nematodes. In most cases, there was no need for special application equipment as well as there was also no need for masks or specialized safety.

Vyas *et al.* (2004) reported the effect of ij's of *Steinernema riobravae* on root knot nematodes, *M. incognita* and *M. javanica* pt. 2 on okra cv. GOH 1 in pots under net house conditions. *S. riobravae* has suppressive effect against

both the plant parasitic nematode species. Results showed significant reduction in root knot index caused by *Meloidogyne incognita* on okra roots using sequential application of EPN's a week prior to root-knot nematode treatment followed by simultaneous application of both the nematodes. Effects on *Meloidogyne javanica* pt. 2 evaluated in 2003 indicated that sequential application of EPN's a week prior to root-knot nematode treatment had better suppressive effect on plant parasitic nematodes than simultaneous application. The study has indicated that the ij's of *S. riobravae* provided a possible control of plant parasitic nematode larvae on okra.

Shapiro-Ilan *et al.* (2006) investigated suppressive effects of *Steinernema feltiae* (strain SN) and *S. riobrave* (Strain 7-12) applied as infective juveniles and in infective host insects, as well as application of *S. feltiae's* bacterial symbiont *Xenorhabdus bovienii* on *M. Patityla*, a parasite of pecan and walnut. In two separate greenhouse trials, the treatment was applied to pecan seedlings that were simultaneously infested with *Meloidogyne partityla* eggs; controls received only water and *M. partityla* eggs. Additionally, all treatment applications were reapplied (with *M. partityla* eggs) two months later. Four months after initial treatment, plants were assessed for number of galls per root system, number of egg masses per root system, number of eggs per root system, number of eggs per egg mass, number of eggs per gram dry root weight, dry shoot weight, and final population density of *M. partityla* second stage Juveniles (J_2). In the first trial, the number of egg masses per plant was lower in the *S. riobrave* infected host treatment than in the control (by approximately 18%). In the second trial, dry root weight was higher in the *S. feltiae* infected host treatment than in the control (approximately 80% increase). No other treatment effects were detected. The marginal and inconsistent effects observed in our experiments indicate that the treatments applied are not sufficient for controlling *M. partityla.*

Molina *et al.* (2007) studied the effects of live and dead ij's of *Heterorhabditis bacteriophora* JPM4, *H. baujardi* LPP7, *Steinernema feltiae*

SN and *S. carpocapsae* in tomato plants. All were evaluated against eggs and J_2 of the plant parasitic nematode *Meloidogyne mayaguensis*. 100 infective juveniles were applied with 350 eggs, 350 j_2 175 eggs + 175 j_2 to tomato plants. Both experiments lasted 9 weeks, and the variable evaluated was number of galls per plant. When eggs were used for infections in the first trial, plants exhibited lower gall number compared to control when live and dead *H. baujardi* ij and live *S. feltiae* ij were added (9.7, 4.5, 7.3 and 85.7 galls, respectively). In the second trial, live *S. feltiae* and *S. carpocapsae* Ij influenced gall formation compared to control (14.33, 14.57 and 168.02, respectively) when j_2 were used for infections, plants with live *H. baujardi* ij presented less galls when compared to control in both trials (38.3 and 355.7 galls in the first trial and 145.2 and 326.2 in the second one, respectively). Infection with a mixture of j_2 and eggs resulted in fewer galls than when live *S. feltiae* ij were present in both trials, compared to control (38.3 and 44.2 galls vs. 275.3 and 192.2 galls, respectively). Both *H. baujardi* and *S. feltiae* apparently may be inhibiting egg hatching and j_2 infection.

Noweer and Wakeil (2007) studied the effects of combining an entomopathogenic nematode (EPN's) and nematode-trapping fungi to control root-knot nematode in the laboratory and in a tomato field. Bioassay effects of EPN's (*Heterorhabditis bacteriophora*) on growth of the two nematode-trapping fungi (*Dactylaria brochopaga* and *Arthrobotrys comcaides*) attacking J_2 of *Meloidogyne incognita* were studied in the laboratory. A field experiment was conducted in a tomato field. The mortality percentages were higher in combining EPN's and trapping fungi than either by trapping fungi or EPN's alone. Combining EPN's with *A. comcaides* fungi caused mortality higher than application by EPN's and trapping fungi *D. brochopaga* .The highest mortality percentage of combined EPN's and trapping fungi on larvae, root galls and egg-masses of *M. incognita* in tomato field was in the treatment of combined EPN's and *D. brochopaga* several times and the treatment of combined EPN's and *A. comcaides* several times, followed by the treatment of combined EPN's

and *D. brochopaga* one time, and treatment of combined EPN's and *A. comcaides* one time. In the third stage came the treatment of *D. brochopaga* alone, treatment of *A. comcaides* alone and finally the treatment of EPN's alone. The highest tomato yield was recorded in treatments of combined EPN's and *D. brochopaga* or *A. comcaides* compared to the separate treatment and control. Thus, it was recommend to farmers to use combination of EPN's and trapping fungi for increasing the mortality of *M. incognita* in tomato fields.

Vyas *et al.* (2008) studied that native isolated *Xenorhabdus* from *Steinernema* spp. manifest wide variety of secretary proteins, mainly in three clusters having mol. wt. in the range of 20-21, 46-51 and 60-66 KDa. The proteins of high mol. wt., 76-90 KDa, apart from regular proteins are produced only in *Xenorhabdus* isolates of *S. riobrave* (Sr M and A), but not in the isolates of *S. carpocapsae* (Sc) and *S. thermophilum* (St). Under pot culture efficacy, average galls/root of groundnut (root-knot index) was significantly low with higher shoot and root weights in exo- and endo-toxic factor (2%V/W) treatment of different *Xenorhabdus* isolates over control. PCR amplification of genomic DNA showed multiple amplified products of *Xenorhabdus* isolates (Sr A and M, St).

Aleem *et al.* (2009) studied the effect of entomopathogenic nematodes, *Steinernema asiaticum*, *S. glaseri, Heterorhabditis indica* and *H. bacteriophora* on the development of *M. incognita*. Effect of different inoculum levels and time of application, live and dead EPN or root-knot nematode in addition to the persistence in soil was also studied. It was found that suppression of *M. incognita* varied with application time. The high application rates of EPN reduced *M. incognita* infestation as compared to low rates. Reduction in root knot nematode invasion and development was recorded. Numbers of EPN were reduced in non-sterilized soil. Both dead as well as live entomopthogenic nematodes and their material were responsible for lower invasion by *M. incognita* in tomato.

2.10. Biological control of root-knot nematodes by fungal inoculants:

Phytonematodes including root-knot nematodes being regarded as serious pests constituting an important major limiting factor in the production of agricultural and horticultural crops, thus attracting the attention of most nematologists and pathologists who are seriously concerned to manage them. Various chemical, cultural, physical and biological measures are adopted for the management of plant parasitic nematodes. Biological control at present seems to be practically demanding and the most relevant approach due to greater awareness of the essentiality of pollution free environment (Khan, 1990). Biocontrol mainly deals with the application of biological agents for the reduction of plant parasitic nematode population by stimulation of natural enemies of the nematodes like fungi and bacteria in the soil environment.

Biological control aims at increasing the parasites, predators and antagonists of nematode and fungus in the soil ecosystem, in order to increase the mortality of plant parasitic nematode leading to the biological suppression and development of suppressive soils in sustainable manner. This can be done by introduction and augmentation of the biotic component of environmental resistance. The biocontrol agents provide protection against plant diseases either by direct action against the pathogen i.e. antagonism which includes parasitism, antibiosis, competition and/or indirectly by reducing host susceptibility i.e. host mediated interaction and includes exudation, altered rhizosphere, induced resistance, hypovirulence, IPGR etc. (Chaube *et al.*, 2003). Microorganisms have been extensively used for the biological control of soil borne plant diseases as well as for promoting plant growth. The important genera of fungi studied as biocontrol agents are *Trichoderma*, *Paecilomyces*, *Verticillium*, *Glomus* spp., *Gliocladium*, *Aspergillus* etc. Out of these genera, *Paecilomyces*, *Trichoderma*, *Gliocladium*, *Talaromyces* are being extensively studied for their use in biocontrol. Biocontrol agents colonize the rhizosphere,

the site requiring protection and leave no toxic residues as opposed to chemical.

The information on status of biocontrol in natural conditions with regard to the organism associated with the nematodes and their prevalence in field emerging through such studies can be exploited for practical purposes.

Among the microorganisms that parasitize or prey on nematode or reduce nematode population by their antagonistic behavior, fungi and bacteria hold key positions. Fungi continuously destroy nematode in virtually all soils because of their constant association with nematode in rhizosphere. Fungi possessing the capacity to destroy or deleteriously affect nematodes vary considerably in both their biology and taxonomic relationships. They range from obligate, endoparasitic forms, many of which are zoosporic to predaceous trap forming species and opportunistic fungi that colonize reproductive structure such as cysts and eggs. Biocontrol agents particularly nematode destroying fungi are common and abundant in both natural and agricultural soils in all kinds of decaying organic materials (Sayre 1980), Kerry (1975, 1980, 1987), Jatala (1986), Khan and Esfahani (1990), Galper *et al.* (1991), Leiji *et al.* (1992) and Trivedi (1992).

2.10.1. Biological control of Root-knot nematodes with *Paecilomyces lilacinus*

Roman and Rodriguez-Marcano (1985) examined the effect of *P. lilacinus* in the larval and root-knot formation of *M. incognita* in tomato. The fungus controlled the nematodes and reduced root knot formation and thus fewer larvae were found in roots and soil of plants inoculated with the fungus.

Dickson and Mitchell (1985) evaluated *Paecilomyces lilacinus* alone and in combination with ethoprop and phenamiphos for management of *M. javanica* on tobacco. The yield of crop was maximum in the treatment where fungus was present.

Jatala (1986) found that *P. lilacinus* causes egg deformation in *M. incognita* with the help of diffusible toxic metabolites (Jatala, 1986, Jatala *et al.*, 1985). The fungus causes alteration in the eggs cuticular structure by enzymatic activity and helps hyphal penetration. This changes egg shell permeability or causes perforation in the cuticle which allows seepage or free movement of diffusible metabolic compounds.

Rohana *et al.* (1987) reported that *Paecilomyces lilacinus* inhibited the population increase of *Meloidogyne* spp. on tomato at 0.5 and 1.0 g inoculums / 750 g soil.

Hewlett *et al.* (1988) evaluated *Paecilomyces lilacinus* as a biocontrol agent of *M. javanica* on tobacco. The efficacy of *P. lilacinus* alone or in combination with fenamiphos, and ethoprops for controlling *M. javanica* on tobacco cv. NC 2326 and the ability of the fungus to colonize in soil under field conditions were evaluated for 2 years in microplots. Nematicides showed higher yield in both years while reverse was true with *P. lilacinus*. But, the fungal population decreased two folds.

Jimmy and Gallod (1988) evaluated the efficiency of *P. lilacinus* as a biological control agent of *Meloidogyne* spp. viz., *M. javanica, M. arenaria* and *M. incognita* under greenhouse conditions. The fungus infected eggs and females occasionally.

Cabanillas *et al.* (1989) found best protection of tomato against *M. incognita* with 10 and 20g of *P. lilacinus* infected wheat kernels and *P. lilacinus* alone, delivered into soil 10 days before planting. Greatest suppression of egg development occurred in plots treated with *P. lilacinus* in pellet.

Sharma and Trivedi (1989) observed that the number of egg masses and number of eggs/egg mass were much lower in plants treated with *P. lilacinus* as compared with control.

Khan and Esfahani (1990) studied the efficacy of *P. lilacinus* for controlling *M. javanica* on tomato in greenhouse. Root galling and egg mass

production were greatly reduced. The fungus were more effective when both organisms were inoculated simultaneously or the fungus preceded the nematode in sequential inoculation.

Trivedi (1990) evaluated the fungus *P. lilacinus* for the biological control of root-knot nematode, *M. incognita* on *Solanum melongena. Paecilomyces lilacinus* was used to control *M. incognita* under Indian agroclimatic conditions. The fungus was screened on sixteen different cultural substrates. Maximum spore load per gram was found on rice grains. Better plant reductions in gallings, final soil nematode population, number of eggs per egg mass were noted in fungus treated aubergine plants.

Zaki and Maqbool (1991) studied the efficacy of *Pasteuria penetrans* with two soil fungi on the biological control of *M. incognita* on okra. The results revealed that application of biological control agent's viz., *Paecilomyces lilacinus, Talaromyces flavus* and *Bacillus subtilis* when used individually or in combination with *P. penetrans* enhanced plant growth characteristics such as length and weight of okra shoots and significantly reduced root-knot indices on okra plants.

Zaki and Maqbool (1991) grew chickpea seedings in pots inoculated with *M. javanica* alone, simultaneous inoculation of *M. javanica* and *P. lilacinus*, inoculation of *M. javanica* followed by *P. lilacinus* after 1 week and inoculation with *P. lilacinus* followed by *M. javanica* after 1 week. Root-knot indices were greatly reduced by *P. lilacinus* after 60 days, however, the fungus being most effective at 2g/pot one week before inoculation. Soil application of *Paecilomyces lilacinus* at 3 to 5% (W/W) on a neem cake base was useful in controlling *Meloidogyne javanica* on groundnut cv. GC 2, seed treatment though found not to be effective (Patel *et al.*, 1995).

Wali *et al.* (1991) conducted the pot experiment in which *P. lilacinus* cultured on wheat bran (WB) applied either as a soil treatment 10 days before or after sowing at 1g/kg soil (5×10^8 spores), or as seed treatment (3×10^7 spores/seed) or in different combinations to sandy soil infected with *M.*

javanica. Fungus application in general, resulted in better crop growth of okra. The methods (soil/seed treatment) or time (pre/post sowing) and fungus application were found to be equally effective. Wheat bran (WB) alone also promoted shoot length and fresh shoot weight to a lesser extent. Root galling was significantly reduced in fungus treatments. Application of WB alone failed to reduce root galling. The time and method of fungus application was at par, as far as reduction was concerned. Wheat bran proved better than carbofuran (1kg a/ha) in gall suppression.

Khan and Khan (1992) tested 15 different fungal filtrates for their nematicidal properties against *M. incognita*. The percentage mortality and inhibition in hatching of nematodes was directly proportional to the concentration of the culture filtrates. *Nigrospora sphaerica* and *Paecilomyces lilacinus* had maximum nematicidal activity and *Theilavia terricola* the least.

Pandey and Trivedi (1992) applied *P. lilacinus* to *Capsicum annum* seedlings infested with *M. incognita* in a pot experiment. In treated pots there was a decrease in the number of galls, eggs per egg mass, final soil application and hatching of *M. incognita* eggs.

Siddiqui and Mahmood (1992) studied four biocontrol agents viz., *Paecilomyces lilacinus, Acrophilalophora fusisphora, Bacillus licheniformis* and *Alcaligenes faecalis* for the control of *M. incognita* race-3 and *Macrophomina phaseolina* on chickpea. All the biocontrol agents except *A. faecalis* were found more effective in improving plant growth when used against *M. incognita* alone or with *M. phaseolina* but less effective against *M. phaseolina* alone. The biocontrol agent, *P. lilacinus* was the best agent, while, *B. licheniformis* and *A. fusisphora* were equally effective.

Abd *et al.* (1993) observed that *P. lilacinus, T. harzianum* and *Epicocum spp.* reduced the percentage of egg hatching and larval activity of *M. incognita* when compared to the control treatments under laboratory conditions. The reduction was more significant when 2 fungal species were used in combination or higher dosage was used. There was a clear and significant

difference in the efficacy of different antagonistic fungi under greenhouse conditions but no statistical difference was found between *Paecilomyces lilacinus* and *Epicocum* sp. under field conditions.

Ehteshamul Haque *et al* (1994) reported that the use of biocontrol agents viz., *Verticillum chlamydosporium, Paecilomyes lilacincus, Talaromyces flavus* and *Bradyrhizobium japonicum* significantly (P <0.05) reduced root-knot infection on chickpea. Significant reduction in gall formation was observed when *V. chamydosporium* was used with *B. japonicum*. Greater increase in fresh weight of shoot was observed where *T. flavus* was used with *B. japonicum* followed by the use of *B. japonicum* alone or with *P. lilacinus*. Use of *P. lilacinus* alone or *T. flavus* with *B. japonicum* also increased the plant height.

Noe and Sasser (1995) evaluated the efficacy of *P. lilacinus* in controlling *M. incognita* on four vegetable crops and soyabeans under field conditions. Experiments with tomato var. Marglobe, okra var. Clemson spineless, eggplant var. Black Beauty, and pepper var. Yolow won den were carried out in two different sites in wake country, North Carolina. An experiment with soyabeans var. Lee 68 was carried out in a field in Johnston country, North Carolina. The yield of vegetable crops in plots treated with *P. lilacinus* was higher than untreated plots in both experimental sites. Cumulative yield data for okra and tomato showed a wide difference between control plots and plots treated with fungus as the season progressed and differences between treatments increased with each harvest date. Similarly, *M. incognita,* juvenile counts were lower in treated plots versus than in control plots. There were more *M. incognita* juveniles in control plots treated with either *P. lilacinus* or fenamiphos. The fungus provided the same level of nematode suppression as the nematicide.

Khan and Saxena (1996) observed that the use of *P. lilacinus* showed significant control of *M. incognita* followed by *M. javanica, M. arenaria* and

Rotylenchulus reniformis on tomato plants. The egg masses were found more vulnerable to fungal infection than eggs and females.

Zaki and Maqbool (1996) reported that *P. lilacinus* and *V. chlamydosporium* (isolates vc-10 and vc-P) significantly reduced gall formation due to *M. javanica* on chickpea. There was no significant difference between biocontrol agents in reducing gall formation. Shoot mass and plant height significantly increased in treated plants.

Khan and Saxena (1997) reported that root dip treatment with culture filtrates of *Aspergillus niger, P. lilacinus* and *T. viride* was particularly beneficial in reducing *M. javanica* damage on tomato in pot experiments. Of the other 8 fungal species tested, *Alternaria alternata, Curvularia clavata, Penicillum* sp. and *Rhizoctonia solani* had no effect on the nematode population or on plant growth and fruit yield.

Hazarika *et al.* (1997) observed that *Paecilomyces lilacinus* to be effective against root knot nematode in betel vine (Piper bettle). Application of *P. lilacinus* @ 4 g/kg soil, increased the plant growth and yield of betel vine with decreased root knot infestation.

Rao *et al.* (1998) recorded that the use of vesicular arbuscular mycorrhiza, *G. mossaeae* with a nematophagus fungus, *P. lilacinus* for the management of *M. incognita* on eggplant (aubergine). Both biocontrol agents did not affect each other colonization on the roots resulting in an additive effect on the management of *M. incognita.* The final nematode population was significantly less in the treatment where *G. mosseae* and *P. lilacinus* were integrated.

Perveen and Ghaffaar (1998) reported that *Paecilomyces lilacinus* showed complete control of *M. javanica* infection on tomato root in soil containing an inoculum of 2000 eggs/250 g soil. The efficacy of *P. lilacinus* declined where the density of *F. oxysporum* increased to 10^6 C. F.U./g and nematodes were 4000 eggs/250g soil.

Khanna (2000) examined that the parasitic fungus *Paecilomyces lilacinus*, when inoculated in the soil along with eggmass or freshly hatched juveniles of the root-knot nematode, *Meloidogyne incognita*, resulted in improvement of plant growth parameters in aubergine. An inoculum level of 3.6 g fungus per 600 g soil was estimated as the optimum level for keeping the root-knot gall index under check and enhancing growth. The best plant growth and minimum root gall index was achieved when fungus was inoculated 15 days earlier than nematode inoculation but statistically as par with the treatment in which inoculating of fungus and nematode were done simultaneously.

Yang *et al.* (2000) reported that nematophagous fungi, isolated from nematode affected areas in fujian province, china, were found to occupy 36.9% of the area sampled. *Trichoderma* spp., *Gliocladuim roseum* and *P. lilacinus* showed significant efficiency in controlling *Meloidogyne* spp., and promoting in the growth of tomatoes. In laboratory tests, the biocontrol efficiency of the fungi was 82.6%, 75.0% and 82.6%, respectively; while in field tests it was 55.3%, 50.0% and 62.5% respectively. Compared to the control plants, the height of treated tomato plants increased by 5.7 cm, 4.9 cm and 4.6 cm, respectively, after 60 days of cultivation.

Hafeez *et al.* (2001) reported that the addition of *P. lilacinus* and *T. harzianum* as nematophagous fungi separately along with organic substrate to the infested soil sufficiently retarded the pathogenic activity of *M. incognita.* Addition of *P. lilacinus* and *T. harzianum* in combination amended with organic substrate gave the effective control of root-knot nematode population thus reduced root-knot disease and increased plant vigor.

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with organic substrate gave the effective control of root-knot nematodes population and thus reduced root-knot disease leading increased plant vigor.

Dhawan *et al.* (2004) observed the efficacy of oviparasitic fungus, *Paecilomyces lilacinus* against root-knot nematode, *Meloidogyne incognita* on okra. The results showed that the application of *P. lilacinus* as seed treatment @ 10, 15 and 20 g/Kg seed and soil application @ 1.5 and 3% $^{w}/_{w}$ significantly increased plant growth characters and suppressed galls, egg masses/ plant and eggs/egg mass. Of the two types of fungal application, soil application was found to be better in increasing plant growth characters and reducing root-knot nematode population compared to nematode alone treatment.

Debora *et al.* (2005) reported that root-knot disease caused by *M. incognita* is a matter of great concern because it affects several economically important crop plants. The use of solid-state fermentation (SSF) may help to elaborate efficient formulations with fungi to be employed in the biological control of nematodes. Attempts were made to select low-cost substrates for spore production of a strain of *P. lilacinus* with known nematicidal capacity. Coffee husks, cassava bagasse and defatted soyabean cake were utilized as substrates, and sugarcane bagasse was used as support. Formulations were carried out in filter paper at 28^{0}C for 10 days. The products obtained by SSF were evaluated for their nematicidal activity in pot experiments containing one seedling of *Coleus* inoculated with *M. incognita*. The plants were evaluated 2 months after inoculation. Fermented products showed a reduction in the number of nematodes. The best results were obtained with defatted soyabean cake, which showed almost 100% reduction in the number of nematodes; the reduction with coffee husk was 80% and with cassava bagasse was about 60%.

Goswami *et al.* (2006) carried out an experiment to study the effect of two fungal bioagents along with mustard oil cake and root-knot nematode *Meloidogyne incognita* infecting tomato. Bioagents viz., *Paecilomyces lilacinus* and *Trichoderma viride* alone or in combination with mustard cake and furadon promoted plant growth and reduced number of eggs/egg mass. The

fungal bioagents along with mustard cake and nematicide showed least nematode reproduction factor as compared to untreated infested soil.

2.10.2. Biological control of *Meloidogyne incognita* with *Trichoderma harzianum*:-

Windham *et al.* (1986) investigated the effects of *T. harzianum* and *T. koningii* on two commercial maize hybrids grown with and without *Meloidogyne arenaria*. Root necrosis was not observed on plants grown in soil infested with either *Trichoderma* spp., *M. arenaria* or a combination of either fungal isolates with the nematode. Shoot and root growth of both hybrids in *Trichoderma* spp. infested soil was significantly enhanced.

Dos Santos *et al.* (1992) reported that *T. harzianum* was a good egg parasite of *M. incognita* race 3-killing 53% eggs *in vitro*.

Parveen *et al.* (1993) compared *T. harzianum, T. koningii, Gliocladium virens, P. lilacinus, Bradyrhizobium japonicum* and *Rhizobium meliloti* with carbofuran for control of *M. javanica* on tomato and okra in soil naturally or artificially infested with *F. oxysporum*. Artificial infestation of soil with *F. oxysporum* significantly reduced gall formation. *T. harzianum, T. koningii* and *G. virens* showed better control of *M. javanica* in naturally infested soil than in *Fusarium*-infested soil on both plants. The biocontrol agents, *P. lilacinus*, *B. japonicum* and carbofuran significantly controlled gall formation on tomato and okra roots in natural and *Fusarium*-infested soil. *Rhizobium meliloti* controlled *M. javanica* in naturally infested soil in tomato and in both naturally and *Fusarium*-infested soil on okra.

Kowalik (1994) studied rhizosphere fungi of tomato during 2 spring cycle of hydroponic culture with peat substrate, mineral wool and a recirculating nutrient solution. The pathogenic fungi identified were *Cylindrocarpon didymum, Fusarium culmorum, F. oxysporum, Thanatephorus cucumeris* and *V. albo-atrum*. A natural protection from the pathogens was provided by the fungal antagonists *T. aureoviride, T. harzianum, T. piluliferum, T. pseudokoningii* and *T. viride*.

Pathak and kumar (1995) recorded maximum (more than 96%) *M. graminicola* mortality at 100 and 50% concentration of *T. harzianum* culture filtrates.

Ivanova *et al.* (1996) tested seven *Trichoderma* spp. strains and eight *B. thuringiensis* strains, including a microbial pesticide for control of *Meloidogyne* spp. on cucumber and tomato *in vitro*. Liquid overmectin preparations ivomec and vertimec 1.8% and aversect 1.2% were tested in the laboratory and in pot culture. vertimec (0.01-0.1%) gave the best control of *Meloidogyne*, followed by aversect and ivomec. Satisfactory control was achieved with *Trichoderma* strains and microbial pesticide based on *B. thuringiensis*.

Khan *et al.* (1997) showed that *P. lilacinus* at 1.0 g/Kg gave better control against *M. incognita* and *T. harzianum* application alone at 1.0g proved a stronger inhibitor of *F. solani*. *Paecilomyces lilacinus* gave better protection of papaya against *M. incognita* and *F. solani* disease complex than *T. harzianum*. An application of both biocontrol agents limited the damage caused by *M. incognita* or *F. solani* and gave 35% increase in plant growth as compared to individual application.

Sankaranarayanan *et al.* (1998) conducted an experiment to find out the nematicidal effects of the antagonistic fungi. *Trichoderma harzianum* (3 isolates), *T. viride* (2 isolates), *Gliocladium virens* and *G. deliquescent* against *M. incognita* on sunflower. Application of antagonistic fungi to sunflower showed increase in plant height. The maximum plant height (78.7) was recorded in *T. harzianum* ITCC treated pots. Among the fungi tested, *G. virens* was found most effective with the least number of galls and eggmasses on root system and nematode population in soil. *Gliocladium virens* had the potential nematicidal activity against root-knot nematode.

Reddy *et al.* (1998) evaluated the fugal biocontrol agent *T. harzianum, Glomus fasciculatum* and neem cake either singly or in combination (at half the doses) for the management of *M. incognita* infesting tomato under nursery and

field conditions. In the nursery, integration of neem cake with *G. fasciculatum* gave maximum increase in plant growth parameters and the least root-knot index. Root colonization was maximum when neem cake was integrated with *G. fasciculatum* while egg parasitism was highest when neem cake was integrated with *T. harzianum*. Under field conditions, planting of tomato seedlings (raised in nursery beds treated with neem cake + *G. fasciculatum*) in pots incorporated with *T. harzianum* grown on sorghum seeds at 0.5 g per plant was effective in increasing tomato fruit yield and for the management of root-knot nematodes.

Sharma (1999) tested among the eight biocontrol agents i.e. culture filtrates of *V. chlamydosporium*, *P. lilacinus* and *T. harzianum* (strain TH-Z) at 100% concentration caused the highest mortalities of *M. incognita* (77.5%, 74.5% and 69.6% respectively) by the end of the 75 hour exposure period. *T. viride, T. harzianum* (strain TH-1), *B. subtilis, G. virens* and *Laetisaria arvalis* showed poor nematode larval mortality. In comparison, regby (cadusafos) at 1000 ppm caused 84.25% mortality.

Sankaranarayanan *et al.* (1999) conducted a pot experiment to find out the nematicidal effect of the antagonistic fungi *T. harzianum* (3 isolates), *T. Viride* (2 isolates), *Gliocladium virens* and *G. deliquescens* against *M. incognita* on sunflower. The maximum plant height was recorded in *T. harzianum* ITCC treated pots. Among the nematophagous fungi tested, *G. virens* was found most effective with respect to treated plants having the least number of galls and egg masses on root systems and nematode populations in soil. Root galling was significantly reduced in treatments where the roots were coated with the fungus.

Hafeez *et al.* (2000) conducted a comparative study of chemical and biological control treatments against *M. incognita* on the basis of the number of galls formed on roots per tomato plant. *Paecilomyces lilacinus* and *Trichoderma harzianum* amended with organic substrate resulted in the minimum number of galls (110.4) per plant

which was 70.61% less as compared with the control. The minimum reduction in galls (123.40) was with tenekil which was 67.15% less than the control.

Khan *et al.* (2001) reported that the egg hatching percentage varied with different fungi. Different dilutions of fungal filtrate significantly inhibited hatching of *M. incognita*. Larval emergence was, however, inversely proportional to filtrate dilutions. *P. lilacinus T. harzianum* and *V. chlamydosporium* were more toxic than other fungi as least egg hatching were observed at standard and S/2 dilutions.

Pant *et al.* (2001) conducted a pot experiment to assess the efficacy of *T. harzianum, P. lilacinus* and *A. niger* against *M. incognita* in chickpea (genotype Type-3). Crop growth was best with *P. lilacinus* followed by *T. harzianum, A. niger* and a combination of all 3 biocontrol agents. Maximum reduction of root knot nematode was recorded with *P. lilacinus* followed by a combination of all biocontrol agents, *T. harzianum* and *A. niger*.

Suarez *et al.* (2004) reported that mycoparasitic *Trichoderma* strains secrete a complex set of hydrolytic enzymes under conditions related to antagonism. Several proteins with proteolytic activity were detected in culture filtrates from *T. harzianum* CECT 2413 grown in fungal cell walls or chitin and the protein responsible for the main activity (PRA 1) was purified to homogeneity. The enzyme was monomeric, its estimated molecular mass was 28 kDa (SDS-PAGE), and its isoelectric point 4.7-4.9. The number of hatched eggs of the root-knot nematode *M. incognita* was significantly reduced after incubation with pure PRA1 preparations. This nematicidal effect was improved using fungal culture filtrates, suggesting that PRA1 has additive or synergistic effects with other proteins produced during the antagonistic activity of *T. harzianum* CECT 2413. A role for PRA 1 in the protection of plants against pests and pathogens provided by *T. harzianum* CECT 2413 is proposed.

Sukumar *et al.* (2005) reported that *T. harzianum* THN1 parasitizing the egg masses of root knot nematode *M. incognita* was isolated from galled mulberry roots and evaluated for its potential to control root knot disease. In

pot experiments root galling was reduced and leaf yield increased significantly following soil treatment with *T. harzianum* THN1. The extracts obtained from the soils inoculated with *T. harzianum*-THN 1 drastically inhibited the hatching of nematode eggs and the effect was irreversible even after the eggs were transferred to fresh water.

Sharma and Trivedi (2005) reported that mass-scale multiplication was done on cheaper substrates for application of bioagents in the management of plant-parasitic nematodes and fungal pathogens. The bioagents were isolated from the local field soils. Out of the 13 isolated fungi, most of the isolates of *Trichoderma* spp. that were found antagonistic to *F. oxysporum* f. sp. cumini in dual culture technique, were mass multiplied on cheaper agro wastes. Suitability of 6 substrates was screened and tea waste was found to be best followed by wheat bran and sorghum straw. The genus, *T. harzianum* (TS) had the maximum spore load per gram (SLPG) value on tea waste followed by *T. hamatum* (T 16) on wheat bran. Three isolates of bacteria viz., *Bacillus subtilis*, *P. fluorescens* and *Rhizobium* spp. were multiplied on nutrient broth; king's B broth and yeast extract mannitol broth, respectively.

Pandey *et al.* (2006) observed the management of root-knot nematode through the combination of two fungal bioagents, *Aspergillus fumigatus* and *Trichoderma harzianum* both collected from egg mass of root-knot infected brinjal plants. It is attributed that in this treatment the toxic effect of *A. fumigatus* killed a good number of infective juveniles of root-knot nematode in the rhizosphere while *T. harzianum* parasitized the eggs resulting in very poor nematode population. Such integration would prove better as compared to either of the biogents used alone.

Dababat *et al.* (2006) tested *T. harzianum* and *T. viride* for their capacity to reduce the incidence of the root-knot nematode *M. incognita* on tomato. *In-Vitro* studies demonstrated that all tested isolates were effective in causing nematode mortality compared with the control. *Trichoderma* slightly reduced nematode damage to tomato *in vivo*. Treatment of the soil with the biocontrol

agents before transplanting, improved control over treatment directly at transplanting. The *Trichoderma* isolates could not be re-isolated from the endorrhiza, but were successfully re-isolated from the rhizosphere 45 days after fungal inoculation. Only slight increase in plant growth could be measured. The mutualistic endophyte *F. oxysporum* 162, used as positive control, was more effective in root-knot nematode biocontrol than the *Trichoderma* isolates.

Sharma *et al.* (2006) reported that organic materials such as neem oil smearing (1% w/w) of seed and soil amendment with *Datura stramonium* and *Calotropis procera* (Leaves containing 5% alkaloids like Scopolamine/Hyocin) alone and in combination with Kalisena (a commercial formulation of *Aspergillus niger*) bioagent and biofertilizer, AN 27 SD egg parasitic and toxic producing facultative fungi and *Trichoderma harizanum* as okra seed treatment (1% w/w) against *M. incognita* showed that *Calotropis* sp. and neem oil alone mitigated root knot galls by 62%. In combined application, reduction in nematode multiplication was not enhanced than the alone treatments. Neem oil, *T. harzianum* and *Calotropis* in combined application affected maximum galling by over 58% while kalisena in the same combination was not so effective in reducing the number of galls. Plant growth of okra such as shoot length, root and shoot weight was improved in treated soil.

Saifullah *et al.* (2007) reported that root-knot nematode (*Meloidogyne* spp.) is one of the major nematode problems in the vegetable crops especially tomato growing areas of the NWFP. In the present study, efforts were made to find out the efficacy of *T. harzianum* in suppressing the population of root-knot nematodes. Eleven different localities viz., Sakhakot, Dargai, Jaban, Thana, Zarakhella, Parrai, Goratey, Matta, Barikot, Charbagh and Tindodag were surveyed and *T. harzianum* showed strong nematicidal effect against *Meloidogyne* sp. The percentage inhibition of egg hatching was directly proportional to the concentration of the filtrate, 90% in standard solution of culture filtrate, 79% in 1:1 dilution, 31% in 1:10 and 9% in 1:100 dilutions.

Only 5% larval mortality was observed in the sterile distilled water used as control.

Sharon *et al.* (2007) examined the parasitism of *Trichoderma* on *M. javanica* life stages *in vitro*. Egg masses, their derived eggs and second-stage Juveniles (J_2) were parasitized by *T. asperellum*-203, 44, and *T. atroviride* following conidium attachment. *Trichoderma asperellum*-GH 11 attached to the nematodes but exhibited reduced penetration, whereas growth of *T. harzianum*-248 attached to egg masses was inhibited. Only a few conidia of the different fungi were attached to eggs and J_2's without gelatinous matrix, the eggs were penetrated and parasitized by few hyphae, while J_2's were rarely parasitized by the fungi. The gelatinous matrix specifically induced J_2 immobilization by *T. asperellum*-203, 44 and *T. atroviride* metabolites that immobilized the J_2's. Scanning electron microscopy revealed the formation of coiling and appressorium like structure upon attachment and parasitism by *T. asperellum*-203 and *T. atroviride*. All, but *T. harzianum* could grow on the gelatinous matrix, which enhanced conidial germination. A biomimetic system based on gelatinous-matrix-coated nylon fibers demonstrated the role of the matrix in parasitism. All *Trichoderma* isolates exhibited nematode biocontrol activity in pot experiments with tomato plants.

2.11. Management of root-knot nematode by oil cakes:-

Use of plant residues and organic amendment has been recognized as an effective way of achieving substantial population reduction of plant-pathogenic life forms like fungi, bacteria, nematodes etc. (Patrick and Toussoun, 1965). Plant residues or organic amendments have been reported to check the population of the pathogens through a variety of mechanisms (Patrick and Toussoun, 1965, Sayre *et al.*, 1964; Cook, 1977; Sitaramaiah, 1990). Leaves of neem (*Azadirachta* spp.) and their products provide nematicidal and other pesticidal activity. There are indications that quite often during the decomposition process of one or other kind of organic materials an identical mechanism may operate, leading to population reduction of pathogenic

organisms. Schipers and Palm (1973) identified ammonia and its fungistatic role in chitin amended soil. Similarly, there are reports in the literature about the enhanced activity of antagonistic fungi due to soil amendments by various organic material which hampers the infection process of pathogenic fungi, nematodes etc.

Oilcakes are generally rich in manurial ingredients such as nitrogen, phosphorous and potash. Oilcakes take about seven to ten days for decomposition after application. They have been found more effective in moist soils during wet weather than in dry soils during dry weather. Oilcakes like castor, groundnut, cottonseed, mustard, mahua, rapeseed, neem and karanj have been used to control many plant parasitic nematodes though some of them have other uses too.

Several workers have carried out the experiments on the efficacy of oilcakes in controlling root-knot damage on tomato, eggplant, okra and chilli (Singh, 1965; Singh and Sitaramiah, 1996, 1971; Goswami and Swarup, 1971; Srivastava *et al.*, 1971; Alam *et. al.*, 1980; Jatala, 1985 and Sitaramiah, 1990).

Singh and Sitaramiah (1966) claimed that the root knot on tomatoes grown after okra can be checked by residual effect of oilcakes in the same field without further amendments. Similar results were also obtained by Alam *et al.* (1971).

Oilcakes were also found to suppress the root-knot nematode development and population of other parasitic nematodes on vegetables and perennial crops (Khan *et al.*, 1966, 1969, 1973, 1979, 1979; Alam and Khan, 1974; Alam *et al.*, 1977; Siddiqui *et al.*, 1976; Zaiyd, 1977; Sitaramiah, 1978; Bhatnagar *et al.*, 1978; Trivedi *et al.*, 1978; Desai *et al.*, 1979; Singh *et al.*, 1980, 1983; Hasan and Jain, 1984; Jagdale *et al.*, 1985; Alam and Ashraf, 1986; Roa *et al.*, 1987; Acharya and Padhi, 1988).

Seed treatment with neem oil cake caused reduction in nematode galls (Singh *et al.*, 1980; Vijaylakshmi and Goswami, 1986).

Khan *et al.* (1966, 1976) reported that the oilcakes of neem, groundnut, mustard, castor and sesamum were effective in reducing the root-knot nematode population in tomato, okra, brinjal and sugarbeet.

Khan *et al.* (1969) confirmed that suppression of plant parasitic nematode population could be achieved with oil cakes of margosa, mahua and castor.

Srivastava *et al.* (1971) found that sawdust 1080.44 kg/ha and neem cake 486 kg per acre were most effective in reducing gall formation in the trial with neem and castor against *M. javanica* in brinjal and tomato.

Hazarika (1990) tested castor, neem and mustard cakes against *M. incognita* in brinjal crop. Neem cake was found effective to increase plant growth and yield by decreasing root-galling and nematode population.

Mahanta and Phukan (1992) recorded that poultry manure at 2000 kg/ha gave the most effective control of *M. incognita* and increased yield of black gram (*Vigna mungo* var. T_9). Neem and mustard cakes were also effective.

Akhtar and Mahmood (1994) examined that bare root dip treatment of chilli (*Capsicum annum*) seedlings with extracts of decomposed and un-decomposed oil cakes and leaves of neem and castor (*Ricinus communis*) provided protection against root-knot disease caused by *M. incognita*. A curative effect was also noted when roots of pre-infected seedlings were given a similar treatment. Suppression of root-development was greater in pre-infected (Therapeutic use) seedlings than in those inoculated after dip treatment (Prophylactic use). Extracts of decomposed materials were effective than those of un-decomposed ones. Moreover, oil cakes and neem leaves were more effective than leaves of castor.

Joshi and Patel (1995) revealed that all plant products viz., (*Azolla pinnata* R.Br), powder dry leaves of pink and white flower periwinkles (*Catharanthus roseus* L.) and vilayti mendi (*Clerodendron inerme* L.) and cakes viż., castor, karanj, mahua, mustard, neem and piludi fresh and dry *Azolla*, FYM and poultry manure showed improved growth of the crop and also

reduced nematode population in comparison to control treatment with '*Azolla*' was the best as compared to all the other amendments. Higher dose of 3000 kg/ha was more effective than the lower dosages tried.

Roa *et al.* (1997) recorded that neem cake (*Azadirachta indica*) and a biocontrol fungus, *T. harzianum* were evaluated either singly or in combination for the management of *M. incognita* on tomato. Significant increase in plant growth and reduction in root galling and final population of *M. incognita* were observed in tomato seedlings transplanted to neem cake amended soil incorporated with *T. harzianum.* Increase in combination of *T. harzianum* on roots of tomato was also observed in the above treatment which indicated favorable effects of neem cake amendment on the growth of *T. harizanum.*

Ramakrishnan *et al.* (1997) observed that maximum shoot length, fresh and dry shoot weight, root length, fresh and dry root weight and pod yield of okra cv. Pusa sawani, were recorded with the addition of neem (*Azadirachta indica*) cake at 13.5 g/ pot, followed by mustard cake at the same rate. Maximum reduction in number of *M. incognita* females, number of egg masses, number of eggs per egg mass and soil nematode population were also recorded in soil amended with neem.

Goswami *et al.* (1998) conducted a pot experiment to test the comparative efficacy of four oilseed cakes cottonseed, karanj (*Pongamia pinnata*), mahua (*Madhuca longifolia*) and mustard against *M. incognita* infecting okra. A significant reduction in the nematode population was recorded when the treatment with karanj was followed by mustard and mahua. *Aspergillus niger, Fusarium oxysporum* and *T. viride* were observed to be growing on karanj and mustard amended treatment. The culture filtrates of each of the above fungi were highly nematicidal.

Rangaswamy *et al.* (2000) evaluated the efficacy of *Pratylenchus penetrans* and *T. viride* and botanicals (neem and castor cakes) in controlling the root-knot nematode *M. incognita* in tomatoes. *P. penetrans* alone or in combination with neem cake parasitized the nematode juveniles and adults,

whereas *T. viride*, alone or in combination with either neem or castor cakes, was most effective in parasitizing the egg masses of the nematode.

Nagesh *et al.* (2001) studied the application of inorganic fertilizer along with oil-cakes viz., castor and neemcakes were beneficial to the endozoic antagonistic fungus *P. lilacinus* and also enhanced the antagonistic potential of *P. lilacinus* against root-knot nematodes (*M. incognita*) under nursery conditions.

Randhawa *et al.* (2002) conducted *in vitro* studies on larval emergence from eggs of *M. incognita* revealed inhibitory effect of neem cake and rakshak gold (neem based product). Plant growth was increased along with significant reduction in root-knot infestation in pot experiments when seed was treated with botanical extracts. In field experiments, application of neem cake @ 5t/ha decreased root-knot infestation significantly and increased okra yield.

Ahmad and choudhary (2004) used four organic amendments viz., sawdust, manure, mustard cake and neem cake to manage root-knot nematode *Meloidogyne incognita* in French bean. Poultry manure applied as spot application was found to be effective in increasing plant growth characters where as neem cake, as spot application, was found effective in reducing galls, gall masses and soil population of *M. incognita*. Spot application of all the organic amendment was comparatively better than furrow application in reducing nematode population and increasing yield.

Tiyagi and Ajaz (2004) found that addition of organic matter to soil such as oil seed cake of neem (*Azadirachta indica*), castor (*Ricinus communis*), groundnut (*Arachis hypogea*), linseed (*Linum usitatissimum*), sunflower (*Helianthus annus*) and soyabean (*Glycine max*) were found to be highly effective in reducing the multiplication of soil nematode and subsequently increased plant growth. The multiplication rate of nematodes was less in the presence of *P. lilacinus* as compared to the absence of *P. lilacinus*. Most effective combination of *P. lilacinus* was with neem cake, under filed conditions.

Pandey *et al.* (2005) conducted an experiment to study the management of root-knot nematode (*M. incognita*) and *Fusarium* wilt (*F. oxysporum* f. sp. *ciceris*) or chickpea cv. H-108. The treatments comprised *G. fasciculatum*, *T. viride*, *P. lilacinus*, neem oil seed cake, *G. fasiculatum* + *T. viride*, *G. fasciculatum* + *P. lilacinus*, *G. fasciculatum* + neem oil seed cake, *Glomus fasciculatum* + *T. viride* + *P. lilacinus* + neem oil seed cake. They observed that the highest shoot, root length, root and shoot weight, chlorophyll content, number of bacterial nodule, number of pods per plant, percentage colonization of VAM, chlamydospore population of *G. fasciculatum* per 100g of soil was obtained with *G. fasciculatum* + *T. viride* + *P. lilacinus* + neem oil seed cake.

Goswami *et al.* (2006) studied the effect of two fungal bioagents along with mustard oil cake and furadan against root knot nematode *M. incognita* infecting tomato under greenhouse condition. Bioagents viz., *P. lilacinus* and *T. viride* alone or in combination with mustard cake and furadan promoted plant growth, reduced number of galls/plant, egg masses/root system and eggs / egg mass. The fungal bioagents along with mustard cake and nematicide showed least nematode reproduction factor as compared to untreated infested soil.

Sreenivasan (2007) investigated the effect of a plant growth promoting rhizobacterium, *Pseudomonas fluorescens*, egg parasitic fungi, *Paecilomyces lilacinus* neemcake and marigold intercrop in different combinations on *M. incognita* and on tuber yield of *Coleus forskohlii*. The results revealed that *Coleus* cutting dipped in 0.1 percent *Pseudomonas fluorescens* at planting with soil application of neemcake @ 400 kg/ha + growing marigold (*Tagetes erecta*) as intercrop, uprooted and incorporated with soil at the time of earthing up (60-70DAP) reduced the root-knot nematode population by 72.0 percent and this treatment was at par with cutting dip with 0.1 percent *P. fluorescens* + marigold intercrop which reduced the nematode population by 69.5 percent. These treatments were equally effective in increasing the root-tuber yield by 22

percent. However, the C: B ratio was higher in *P. fluorescens* + marigold intercrop than other.

2.12. Management of root-knot nematode by chemical nematicides:-

Chemicals have been considered as the most effective and efficient means of managing the pathogenic population of fungi, bacteria, nematodes etc., and chemicals such as nematicides, fungicides and herbicides have been formulated and are being successfully used against their respective targets (Van Gundy and Mc Kenry, 1977; Dekker, 1977; Wright, 1981). Soil fumigants have been used singly or in combination against pathogens involved in complex disease. Usually combination of fumigants have been found to have chemical compatibility coupled with toxicity against soil biota like insect pests, fungi and weeds capable of causing sustainable damage.

Reddy (1975) examined that spot and row application of aldicarb 10G, fensulphathion 5G or carbofuran 3G increased the yield of tobacco infested with *M. incognita*.

Davide (1979) observed the efficacy of aldicarb 10G, mocap 10G, furadon 3G, vydate, nalite and nemagon 20G on tomato against *M. incognita* and vydate was found to be most effective.

Kheir *et al.* (1983) observed the efficacy of aldicarb, carbofuran, ethoprop, fensulafathion, miral and oxamyl in controlling the root-knot nematode, *M. javanica* on okra. All nematicides tested reduced nematode population. Miral showed the highest percentage of reduction, while carbofuran achieved the lowest one. All chemically treated plants grew better than untreated plants. In general all nematicides used in this study minimized the root-gall index values of treated plants to a greater extent.

Reddy (1983) tested aldicarb, carbofuran, phorate and aminophos to the control of *M. incognita* in tomato nursery and found aldicarb and carbofuran effective to control the nematode population.

Dickson and Mitchell (1985) evaluated *Paecilomyces lilacinus* alone and in combination with ethoprop and phenamiphos for management of *M. javanica* on tobacco. The yield of crop was maximum in the treatment where fungus was present.

Paruthi *et al.* (1987) pointed out that there was significant improvement in plant growth characters and reduction in number of galls and egg masses, when su-babool leaves were supplemented with carbofuran.

Pandey and Trivedi (1990) tested three systemic chemicals, viz. vegfru foratox 10G, vegfru diafuran 3G and rugby 10G at preinocultation stage to control *M. incognita* infestation. All the chemicals in every dose could control disease occurrence and further multiplication of pathogen to varying degrees, best results were observed with application of rugby 10G (0.7kg a.i./acre) followed by diafuran 3G (1.2kg a.i./acre) rugby 10G (0.5 kg a.i./acre). Reduction of root-knot index from 6.91-3.19 was observed when plants were treated by rugby 10G. Final soil population and reproduction factor was also reduced from 2810 and 2.81 in control to 1068 and 1.06 in pots treated with rugby 10G.

Jain and Gupta (1992) tested tomato plants with carbofuran, fenamiphos *diazionon*, phorate or ethoprephas and aubergine plants were treated with chemicals like fenamiphos, triazophas, carbofuran, isofenaphos and leaves of *calotropis procera* or *Ricinus communis* for control of *M. javanica*. Tomatoes and aubergines treated with fenamiphos at 0.6 and 0.4 g/m^2 respectively had the lowest number of galls.

Akthar and Farzana (1993) used aldicarb, carbendazim, carbofuran, ethoprophos and ebufos @ 4kg a.i/ha and oilcakes of linseed, mustard and neem @ 1g /kg soil for the management of *M. incognita* parasitizing *Trachyspermum ammi* (L.). In general, all the treatment were able to reduce the root-knot development and nematode population and to increase the plant fresh and dry weight. The best results in term of reduction in root-knot index and improvement in plant growth were achieved by treating plants with

ethoprophos followed by carbofuran, neem cake, carbendazim, ebufos, mustard and linseed cake.

Ehteshamul-Haque *et al.* (1995) reported that *Verticillium chalamydosporium* was found more tolerant against *carbendazim*, PCNB, benomyl, topsin-M and capton as compared to *P. lilacinus* and *Verticillim chlamydosporium*, benomyl and topsin- M were found more effective than carbofuran in the control of *M. javanica* on okra. *V. chlamydosporium* and *P. lilacinus* also significantly ($P<0.05$) reduced the infection of root infecting fungi viz., *M. phaseolina*, *R. solani* and *F. solani*, as compared to untreated control. Combined use of benomyl with topsin-M was found more effective in controlling *M. javanica, M. phaseolina* and *F. solani*, than use of carbofuran with fungicides viz., carbendazim, PCNB, benomyl, topsin-M and capton. All the fungicides reduced the efficacy of biocontrol agents in controlling root-rot and root-knot infection on okra.

Stephan (1995) observed that the nematicides and horse manure significantly increased the yield and reduced the infection on tomato and eggplant. Cadusafos (6 g/m^2), liquid fenamiphos (5cc/m^2) and dichlofenthin (20 g/m^2) were the most effective, followed by horse manure. Oxamyl and carbofuran, at concentrations of 3 cc/l water and 15 g/m^2, respectively provided some nematode control and increased yields.

Zaki and Maqbool (1995) observed that the use of nematicides viz., rugby, tenekil and furadan significantly reduced root-knot infestation on tomato plants. Maximum reduction in gall formation was produced where rugby was used @ 0.03 g/kg followed by rugby @ 0.02 g/kg, tenekil @ 0.0181 g/kg and Furadan @ 0.12 g/kg soil. Great increase in fresh weight of shoot was observed where rugby was used @ 0.03 or 0.02 g/kg soil.

Vyas *et al.* (1996) examined the effect of two nematicides phenamiphos and carbofuran and a parasitic fungus, *P. lilacinus* were tested alone and in combinations for management of root-knot nematodes on chickpea. The results showed significant differences due to various treatments for fresh shoot weight,

root-knot index and grain yield. Phenamiphos @ 1kg ha^{-1} was found most effective followed by *P. lilacinus* @ 1 t ha^{-1} based on neem cake treatment. However, integration of nematicide and biocontrol agent did not show any additive effect.

Stephan *et al.* (1998) found that cadusafos (rugby) almost totally suppressed nematode gall rating (1.3 and 1.8) producing a higher yield of tomato and eggplant followed by ethoprophos (mocap). *Trichoderma harzianum*, *P. lilacinus* and *Acremonium butyri*. Maximum nematode gall rating (5 on both hosts) was observed in control with the lowest yield in tomato.

Khan *et al.* (2000) compared the chemical and biological control treatments against *M. incognita* on the basis of the number of galls formed on roots per tomato plant. *Paecilomyces lilacinus* and *Trichoderma harzianum* amended with organic substrate resulted in the minimum number of galls (110.4) per plant, which was 70.61% less as compared with the control. The minimum reduction in galls (123.40) was with tenekil, which was 67.15% than the control.

Singh and Goswami (2001) showed the effect of root-knot nematode and wilt-fungus *Fusarium oxysporum* on cowpea in presence of neem cake and chemical pesticide carbofuran. They showed synergetic effect of neem cake and carbofuran on reduction of root-knot nematode infection.

Adekunle and Fawale (2002) evaluated chemical and non-chemical pesticides to manage root-knot nematode *Meloidogyne incognita* under field condition. They emphasized the use of both pesticides in combination for maximum reduction of losses due to root-knot nematode.

Mishra *et al.* (2003) evaluated biopesticides (Neem seed powder, neemark, latex of *Calotropis procera*), chemical (diamethoate, trizophos, chlorophyriphos and carbofuran) and bioagents (*Paecilomyces lilacinus, Aspergillus niger, Trichoderma viride*) against plant parasitic nematodes associated with chickpea effectively controlled the root-knot nematode

infestation as exercised from the observation recorded at the harvest of the crop on population buildup of nematodes, plant growth paramaters and grain yield of chickpea. Seed treatment with carbofuran @ 2% w/w was found equally effective as soil application of neem seed powder but plant growth parameters were better under neem seed powder treatment. *Trichoderma viride* could reduce the nematode population to almost the same extent as observed in other bioagents. Seed treatment with dimethoate @ 1% was found to be least effective in controlling root-knot nematode as compared with other treatment.

Senthikumar and Ramakrishnan (2004) studied the effect of *P. fluorescens, T. viride* and carbofuran 3G independently and in combination for the management of *M. incognita* in okra var. C_o3 under glasshouse conditions. The independent application of *P. fluorescens* (2.5 kg ha^{-1}) and in combination with carbofuran 3G significantly improved the plant growth *P. fluorescens* (1.25 kg ha^{-1}) + carbofuran (0.5 kg a.i.ha^{-1}) treated plant recorded the highest fruit weight / plant (184.48%). While the highest number of colony forming units of *P. fluorescens,* and *T. viride* was recorded in individval application of the same compared to combined application.

Haseeb and Kumar (2005) determined the effect of bio-inoculants viz., *Aspergillus niger*, *Paecilomyces lilacinus*, *Trichoderma harzianum* and *Pseudomonas fluorescens* @ 50 kg/ha each containing 10^8 cfu/g culture, organic amendment materials viz., neem seed powder @ 100 kg/ha fresh leaves of neem (*A. indica*) and Murraya (*Murraya koenigii*) @ 300 kg/ha each, farmyard manure and mint (*Mentha arvensis*) manure @ 1500 kg/ha each, and pesticides viz., carbofuran, topsin-M @ 2 kg a.i/ha each for the management of *M. incognita* @ 4000 j_2/4kg soil) and *Fusarium solani* @ 10g/4 kg soil disease complex of brinjal cv. Pusa kranti. Results revealed that all the treatments significantly improved the plant growth and reduced the percent root infection and root knot index as compared to untreated inoculated control except the treatment with farmyard manure.

Sharma and Haseeb (2006) determined the comparative efficacy of chemicals viz., carbofuran and bavistin each @ 1 mg a.i./kg soil, organic amendments viz., *Azadirachta indica* seed powder, *A. indica* cake each @ 50 mg/kg soil, *Murraya koenigii* leaves powder @ 500 mg/kg soil and mint manure, farmyard manure each @ 1500 mg/kg soil, fungal bioagents viz., *Trichoderma harzianum, T. virens, Paecilomyces lilacinus, Aspergillus niger*, and bacterial bio-agents viz., *Pseudomonas fluorescens* each @ 50ml suspension (2 X 10^8 spores / ml) /kg soil against *Rhizoctonia solani* on *Mentha arvensis*. All the treatments except F.Y.M., significantly improved the plant growth and soil yield of the plants as compared to untreated un-inoculated plants. Analysis of data showed that bavistin was most effective in improving growth, oil yield of the plants as well as in suppression of disease development, followed by *A. indica* seed powder, *A. indica* cake, *T. harzianum*, *P. fluorescens* and *T. virens,* respectively.

Saikia *et al.* (2007) studied the effect of organic amendments viz., neemcake, vermicompost, neem seed kernel sawdust and carbofuran 3G against root-knot nematode, *Meloidogyne incognita* in brinjal under field condition by applying individually and in combinations. All the treatments showed significant effect on plant growth parameters and yield of brinjal with corresponding decrease in the nematode populations both in soil and roots. Among all the treatments, the treatment with neemcake + carbofuran 3G showed superior control in respect of plant growth parameters and yield of brinjal and decrease in nematode multiplication.

Oyekanmi *et al.* (2007) studied the effects of soyabean inoculation with arbuscular mycorrhiza fungus *Glomus mosseae* (200 spores / plant), the nodulating bacterium *Bradyrhizobuim japoicum* (10^6 cells / plant) and the nematode antagonistic fungus *T. pseudokoningii* (6.8×10^7 spores / plant). Application of the microorganisms separately, in dual, or in triple combinations were assessed in the presence of the plant-parasitic nematode, *M. incognita* under green house (1000 J_2 / plant) and field (1500 eggs / plant) conditions,

with two soyabean genotypes. The treatments were compared with application of a synthetic nematicide (furadan 3G® [a.i. carbofuran]), an untreated control without nematodes and a nematode-only control. Application of the microorganisms in full factorial combinations suppressed nematode reproduction in most cases and reduced nematode galling compared to the nematicide treatment.

Chapter 3

MATERIALS AND METHODS

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1. Rearing of larvae of rice moth (*Corcyra cephalonica*):-

The maize grains were heat sterilized in an oven at 100^0C for 30 minutes and then crushed into pieces. About 2 kg broken grains were put in plastic container (5 litre capacity) and treated with 0.1% formalin to prevent the growth of mould. About 1.0 cc *Corcyra cephalonica* (Stainton) eggs were mixed with this and perforated lid was secured and kept in dark for about a month. The fully grown larvae were utilized for both isolation and mass multiplication of EPN's. Some of them were left for emergence of moth which were collected in ovi- position container, containing folded paper strips which are hanged, serving as dark area for hiding and substrate for egg laying.

2. Collection of soil samples and isolation of EPN'S from soil by baiting:-

2.1. Collection of soil samples from Aligarh and isolation of EPN's from collected soil samples by baiting:-

About 195 soil samples were collected from various locations in Aligarh, by random sampling technique during the March-April of 2009. Two to five sub-samples were collected from 10-15 cm layer of soil from the root zone of different plants of each locality and bulked to make composite samples. From this composite sample, 500g of soil was finally taken for baiting. Samples were placed in small plastic containers and each was baited with 5 late instar *Corcyra cephalonica* larvae (first baiting) followed by 3 larvae (second baiting). The containers were covered tightly with lids containing small holes

to facilitate gaseous exchange and kept at room temperature (30-35^{0}C). Larval mortality was recorded daily upto 10 days. The *Corcyra* larvae infested with *Steinernema* sp. became flaccid and their colour changed from white to yellow brown, in case of *Heterorhabditis* sp. the colour changed from white to brownish red or brick red. (Tabassum *et al.*, 2005). The EPN's isolated were then used for mass multiplication and used for further experiments.

2.2. Collection of soil samples from Pulwama district of Jammu and Kashmir and isolation of EPN's from collected soil samples by baiting:-

About 270 soil samples were collected from various locations in district Pulwama, by random sampling technique during May-June of 2009. Two to five sub-samples were collected from 10-15 cm layer of soil from the root zone of different plants of each locality and bulked to make composite samples. From this composite sample, 500 g of soil was finally taken for a baiting. Samples were placed in small plastic containers and each was baited with 5 late instar *Corcyra cephalonica* larvae (first baiting) followed by 3 larvae (second baiting). The containers were covered tightly with lids containing small holes to facilitate gaseous exchange and kept at room temperature (20-25^{0}C). Larval mortality was recorded daily upto 10 days. The *Corcyra* larvae infested with *Steinernema* sp. became flaccid and their colour changed from white to yellow brown, in case of *Heterorhabditis* sp. the colour changed from white to brownish red or brick red (Tabassum *et al.*, 2005). The EPN's isolated were then brought to plant nematological laboratories in A.M.U., Aligarh, were they were mass multiplied and used for the further experiments.

2.3. Collection of soil samples from Shopian district of Jammu and Kashmir and isolation of EPN's from collected soil samples by baiting:-

About 255 soil samples were collected from various localities in district Shopian of Kashmir, by random sampling technique during August-September of 2009. Two to five sub-samples were collected from 10-15 cm layer of soil from the root zone of different plants of each locality and bulked to make composite samples. From this composite sample, 500 g of soil was finally taken for baiting. Samples were placed in small plastic containers and each was baited with 5 late instar *Corcyra cephalonica* larvae (first baiting) followed by 3 larvae (Second baiting). The containers were covered tightly with lids containing small holes to facilitate gaseous exchange and kept at room temperature (20-25^{0}C). Larval mortality was recorded daily upto 10 days. The *Corcyra* larvae infested with *Steinernema* sp. became flaccid and their colour changed from white to yellow brown, in case of *Heterorhabditis* sp. the colour changed from white to brownish red or brick red (Tabassum *et al.*, 2005). The EPN's isolated were then brought to plant pathological and nematological laboratories in A.M.U., Aligarh, were they were mass multiplied and used for experiments to control root-knot disease in brinjal.

2. Mass multiplication of entomopathogenic nematodes (EPN's):-

For experimental purposes, EPN's were mass multiplied *in vivo* on *Corcyra cephalonica* by using White trap method (White, 1927). *Corcyra* larvae were inoculated with EPN on a Petridish lined with filter paper. After 2-5 days, infected insects were transferred to the white trap. This method consists of a dish on which the cadavers rest on inverted watch glass, surrounded by water. The central dish containing the cadavers provides moist surroundings for the EPN emergence from cadaver. New progeny of infective juveniles that emerged from cadaver migrated to the surrounding water where they were trapped and subsequently harvested. This method has the advantage that the ij's migrate away from host cadaver on emergence and continue to do so until the body contents of the host are consumed. EPN's were then separated from the

cadaver using the Baermann funnel technique. Using this rearing technique each *C. cephalonica* larva can yield about 1,50,000 to 2,00,000 infective juveniles. Before their further use or storage, the infective juveniles were separated from the host tissues, dead or live adult nematodes and their symbiotic bacteria. This was achieved by storing the freshly collected nematodes in water at 5^0C for 2-3 days and then washed in plenty of water by decanting and sedimenting.

4.0. Collection of Nematode Inoculum (*Meloidogyne incognita)*:-

Roots of brinjal (*Solanum melongena)* infected with root-knot nematode were collected from brinjal plants. Root-knot nematode species were identified on the basis of the characteristics perineal patterns.

4.1. Mass culturing of root-knot nematode (*Meloidogyne incognita)* for Inoculations:-

Mass rearing of *M. incognita* was done on the roots of susceptible eggplants cv. pusa purple long in 8 inch earthen pots containing about 4 kg soil. One month old seedlings were inoculated with second stage juveniles of *M. incognita*. The purpose of the mass culturing of *M. incognita* was to get the regular supply of the inoculums for the experiments.

4.2. Pure culturing, Raising, Preparation and Maintenance of Inoculums:-

A single egg mass of *M. incognita* was surface sterilized in 1:500 solution of chlorax (calcium hypochlorite) for five minutes and washed thrice in sterilized distilled water. The eggs in the egg mass were allowed to hatch out at 28 ± 2^0C under asceptic conditions in a sieve layered with tissue paper and kept in a Petridish containing sufficient amount of sterilized distilled water. Seedlings of brinjal raised in 8-inch clay pots, containing sterilized soil were

inoculated with the juveniles so obtained. Nematodes were extracted from the soil after 2 months, according to Cobb's sieving and gravity method followed by Baermann funnel technique (Southey, 1970).

The egg masses from heavily infected roots of brinjal on which pure culture of *M. incognita* multiplied were handpicked with the help of sterilized forceps. These egg masses after being washed in distilled water were placed in a sieve with a layer of double tissue paper. The sieve was placed over Petridish (10cm in diameter) containing water. The water level was kept such that it just touched the lower portion of the sieve having egg masses. A series of such assemblies was kept to obtain large number of J_2 required for inoculations in the experiments. After every 24 hours, the hatched out larvae were collected along with water from Petridish in a beaker and fresh water added to the Petridish.

4.3. Counting and standardization of root-knot nematode (*M. incognita*):-

The water suspension of *M. incognita* as obtained above was thoroughly stirred for making homogenous distribution of nematodes before taking 2 ml of suspension in the counting dish for counting the numbers of nematodes under the stereoscopic microscope. An average of five counts was taken to determine the density of nematodes in the suspension. Volume of water in the nematode suspension was so adjusted that each ml contained about 100 nematodes, which was done by either adding more water or decanting the excess amount of water so that 10 ml of this suspension contained 1000 J_2 of *M. incognita*.

5. Preparation of soil for pots:-

The soil was sterilized before it was transferred to 8 inch earthen pots. The pots were filled with 4 kg sterilized soil each pot.

6.1. Collection of Brinjal seeds:-

The brinjal seeds were obtained from local market in Aligarh. The pusa purple long variety of brinjal was obtained. Care was made for seed germinating capacity. Seeds were not treated with any sort of chemical. Plants were first grown in sterilized soil in earthen pots of 8 inch.

6.2. Raising Brinjal Seedlings:-

Brinjal variety pusa purple long was raised in sterilized soil. They were allowed to grow for three weeks.

6.3. Transplanting of Seedlings:-

The three week old Brinjal seedlings grown in 8 inch pots were shifted to another set earthen pot of same size containing same weight of sterilized soil. One day before transplanting, the pots were not watered for the hardening purpose. Seedlings were removed from pots carefully and then adhering soil was removed gently by shaking and then planted in pots. Before transplanting, roots of nursery seedling were not washed to avoid the risk of root damage.

6.4. Inoculation with nematode (*M. incognita)*:-

The nematodes so obtained were used for inoculating experimental pots containing fresh egg plant seedlings grown in 8 inch earthen pots containing 4 kg sterilized soil. Holes of 5-7 cm depth around the plants, with a radius of 2 cm from the plant, were made in which required number (500 *M. incognita* J_2 per 500g soil) were transferred with the help of sterilized pipette. The holes were then plugged with sterilized soil.

6.5. Maintenance of Plant Material:-

Plants were maintained using good maintenance practices. During the course of experiments, plants were watered carefully (not excessively watered, overhead watering of pots was avoided) eliminating risk of leaching nematodes out of the soil or drying out, especially for the first few days after nematode inoculation. One week after germination the seedlings were thinned

keeping single seedling/pot. Before conducting any experiment, plants were always chosen for their uniformity in terms of height (measured from base to last fully expanded leaf) and size of root system. One day before transplanting, the pots were not watered to ensure sufficient hardening of seedling. Seedlings were removed from pots carefully and then adhering soil was removed gently by shaking. Before transplanting, the roots of nursery seedlings were not washed to avoid the risk of root damage. Plants were not watered the day before harvest, facilitating washing out root systems the following day. At the time of termination, lose soil was first shaken of before soaking the roots in water for a few minutes. Having washed off the bulk of soil, the roots were then transferred to a tray of shallow water and the remaining soil particles were washed out using a jet of water or picked out by hand. Once washed, clean roots were kept in a beaker of water until further processing. Experiments were carried during March-April, months of 2009. Running experiments during June and July months were avoided as potted plants and soil in the pots could reach at extremely high temperatures (e.g.45- 50^0C in the afternoon). Experiments were also avoided in the month of December and January as the temperature became very low which is thought to be unfavorable for both entomopathogenic nematodes and *Meloidogyne* spp. Low temperature and reduced light availability could cause slow plant growth

7. Counting and standardization of entomopathogenic nematodes:-

To test inoculum density, the nematode suspension was poured into a measuring cylinder and mixed thoroughly by blowing with pipette. One ml from the original concentration was diluted by adding 3 ml water and counted under stereomicroscope. Total number of nematodes was counted based on the average of 3 counts multiplied by the total volume. Efforts were made to get the clear suspension by dilution. Nematode suspensions were diluted to a concentration of approximately 5,000 ml and were kept in shallow plastic

containers with lids taking care that the suspension is no more than 1 cm in depth to ensure sufficient oxygen availability until formulation. EPN's were stored at 10-15^{0}C. Nematodes were always checked for viability before starting experiments. Nematodes were provided oxygen by aerator used for fish aquarium tanks. Only freshly hatched third stage juveniles (24-48 hours old) of entomopathogenic nematodes, those freshly produced *in vivo* (less than 2 weeks old) were used. The *in vivo* produced EPN's could be kept for 3 months. All stock nematode cultures were recultured every 4 months. The number of *M. incognita* used as inoculum in experiments always refers to second stage infective juveniles of *Meloidogyne incognita.*

8.1. Need for formulation of EPN's:-

Although the infective juveniles of entomopathogenic nematodes can be stored for several months in water in refrigerated bubbled tank, its high cost and difficulties in maintaining quality restraints the deployment of this method. Therefore, nematodes are usually formulated into solid or semisolid liquid substrates soon after they are produced. Several commercial formulations of EPN's are available world wide and easily acceptable in high value niche markets.

8.2. Formulations of EPN's in calcium alginate capsules and their application to soil environment:-

A solution of gel matrix was prepared as per Kaya and Nelsen by dissolving 2g of sodium alginate in 100ml of water and blended for 4-5 minutes. Drops of this solution when placed into a 100mm solution of $CaCl_2$. $2H_2O$ (the complexing solution) formed discrete capsules of calcium alginate. Nematodes, viz. *Steinernema* sp. and *Heterorhabditis* sp. were placed into solution of alginate and then dripped into the complexing solution which was being continuously stirred. Capsules were allowed to complex for 20-30

minutes and then separated from the complexing solution by sieving, rinsed in deionized water and stored at a temperature of 5^0c and 25^0c.

Survival of encapsulated nematodes was checked every 15 days by dissecting samples of five capsules. The nematodes encountered in the microscopic field were examined to determine whether they were alive or dead.

Alginate capsules were applied to experimental earthen pots (soil environment) at various concentrations. When the alginate capsules were placed in soil with adequate moisture, most ij's migrated out of the granules within a week.

Experiment:-

9. Effect of different inoculum levels of EPN's (*Heterorhabditis* sp. & *Steinernema* sp.) on the plant growth parameters of eggplant:-

Culture of *Steinernema* sp. and *Heterorhabditis* sp. was maintained on *C. cephalonica*. EPN's were then formulated on calcium alginate capsules. Both *Steinernema* sp. and *Heterorhabditis* sp. were applied @ 50, 500, 1000, 2500, 5000, 10,000 and 20,000 1js/500 g soil to three week old seedlings grown in 8 inch pots containing 4 kg soils. Un-inoculated plants were kept as control. After 60 days from the treatment application time, they were removed from the pots and the roots balls were shaken until the most of the soil was dislodged from the root. Plant growth parameters such as root length, shoot length, shoot dry weight and root dry weight, number of flowers and weight of fruits were recorded.

Plant dry weight estimation:-

For measuring dry weight, the plants were first kept in oven at $60c^0$ for drying upto 2-3 days. After cooling, shoots and roots were weighted separately.

Experiment:-

10. Management of *Meloidogyne incognita* infecting eggplant by using different inoculum levels of *Steinernema* sp. at concomitant and sequential application times:-

Culture of *Steinernema* sp. was maintained on *Corcyra cephalonica. Steinernema* sp. were then formulated on calcium alginate capsules. *Steinernema* sp. were applied @50, 500, 1000, 2500, 5000, 10,000 and 20,000 ij's/500g soil to brinjal seedlings one week before the inoculation of 500 *M. incognita*/ 500g soil. Healthy plants (un-inoculated) were kept as control. Brinjal seedlings were also inoculated with *M. incognita*. After 60 days, they were removed from the pots and the root balls were shaken until most of the soil had been dislodged from the root. Then parameters of growth like shoot length, root length, shoot dry weight, root dry weight and number of flowers and weight of fruits were recorded.

One set of treatment where *Steinernema* sp. were applied at the same time with root-knot nematode was also run for 60 days. Plant growth parameters were also measured as above.

In another set of treatment *Steinernema* sp. were applied one week after the *M. incognita* was applied and the experiment was also run for 60 days from the day of *M. incognita* inoculation and in the similar way the growth parameters were recorded.

Plant dry weight estimation:-

For measuring dry weight, the plants were first kept in oven at $60c^0$ for drying upto 2-3 days. After cooling, shoots and roots were weighted separately.

Nematode population estimation:-

For extraction of nematodes, the soil from each treatment was mixed thoroughly and a sub-sample of 200g soil was processed through sieves according to Cobb's sieving and decanting method followed by Baerman's funnel technique. Each suspension was collected in a beaker and volume was made up to 100ml. For proper distribution of nematodes, the suspension was bubbled with the help of pipette and 2ml suspension of each sample was drawn and transferred to a counting dish. The number of nematodes was counted in three replicates for each sample. Mean of three such counting's was calculated and the final population of nematodes/500g soil was determined.

To estimate the nematode population in roots, 1g root from each replicate was macerated with enough water in an electrically operated warring blender for about 30 to 40 seconds. The macerate was collected in a beaker and volume was made up to 100ml. The nematode population was counted as described above.

Reproduction factor (Rf) of the nematode was calculated by the formula $R_f = \frac{P_f}{P_i}$, where P_f=final nematode population and P_i=initial nematode population.

Root-Galling estimation:-

The galling caused by *M. incognita* was estimated by counting the number of galls per root system.

11. Raising and Maintenance of Fungal cultures:-

The fungal biocontrol agents viz. *Paecilomyces lilacinus* and *Trichoderma harzianum* were isolated from the brinjal roots and were further raised on Richard's liquid medium having the following composition.

Potassium Nitrate	100.00g
Potassium dihydrogen phosphate	5.00g
Magnesium Sulphate	2.50g
Ferric chloride	0.02g
Sucrose	50.00g
Distilled water	1000ml

The medium was prepared, filtered through the muslin cloth and sterilized in an autoclave at 15 lb pressure for 15 minutes in 250 ml Erlenmeyer flasks, each containing 100 ml of liquid medium. Small bits of the fungal mycelium of respective fungi were transferred to the conical flasks. Inoculated flasks were incubated at 28 ± 2^0C for about 20 days to allow the growth of the respective fungi to be used for further studies. Pure culture of these fungi was continuously maintained on PDA contained in the test tubes by re-inoculation of the fungus after every 15 days.

12. Preparation of Fungal inoculum:-

After incubating the flasks containing fungal culture for about 20 days, the liquid medium was filtered through Whatman filter paper No. 1, the mycelial mat was washed in distilled water to remove the traces of medium and gently pressed between the folds of blotting paper to remove the excess amount of water. Inoculum was prepared by blending 10g mycelium in 100 ml of sterilized distilled water for 30 seconds in a warring blender(Stemerding, 1964). Thus each 10 ml of this suspension contained 1g of fungus.

13. Inoculation techniques of fungi:-

The seedlings were inoculated with 0.5 g of fungal mycelium per 500g soil.

Roots of seedlings just before inoculation of fungal biocontrol agents, were exposed by carefully removing the top layer of soil and required quantity (0.5g fungus/500gsoil) of fungal inoculum was poured uniformly all around the exposed root using a sterilized pipette. Exposed roots were immediately

covered by the soil properly. Each treatment was replicated five times. Watering was done as and when required.

Experiment:-

14. Effect of biocontrol agents, oil-cakes and nematicide-carbofuran on the growth of eggplants:-

Clay pots of 8inch size were filled with 4kg sterilized soil. Powdered oil cakes of neem were applied to one set of pots at the rate of 5% w/w. To the set of pots powdered oil cakes of soya bean were applied at the same rate. Watering was done daily for a period of one week to ensure proper decomposition of the oilcakes. After waiting period of one week, one month old seedlings of eggplants were transplanted singly to these pots.

To the other set of pots containing the same 4 kg sterilized soil, three week old seedling of eggplants were transplanted singly to these pots. To these pots various sets of treatments were prepared as per schedule given below:-

i) *Trichoderma harzianum* @ 0.5g fungal mycelium per 500g soil,

ii) *Paecilomyces lilacinus* at same rate,

iii) Freshly hatched third stage juveniles of EPN's (*Steinernema* sp.) @ 2000 J_3 per 500g soil,

iv) Carbofuran @0.25g per 500 g soil and

v) 500 *M. incognita* j_2 per 500 g soil were used and watering were done. The uninoculated and untreated pots served as control.

Overall there were seven set of treatments besides control (uninoculated and untreated). These sets of treatments are given as below:-

1. *Meloidogyne incognita*
2. Carbofuran
3. *Paecilomyces lilacinus*
4. EPN's
5. *Trichoderma harzianum*
6. Neem cake

7. Soyabean cake
8. Control (uninoculated and untreated)

Each set of treatments had five replicates.

The experiment was terminated after 60 days of transplantation. The soil from the roots was removed. The plants growth parameters like plant length, dry weight, number of flowers per plant and weight of fruits per plant were recorded.

Experiment:-

16. Management of *Meloidogyne incognita* infecting eggplant by using biocontrol agents, oil-cakes and nematicide-carbofuran alone or in combination with EPN'S (*Steinernema* sp.):-

Clay pots of 8 inch size were filled with 4 kg sterilized soil. Powdered oil cakes of neem and soyabean were applied at the rate of 5% w/w. Watering was done daily for a period of about 1 week to ensure proper decomposition of the oilcakes. After an awaiting period of one week, three week old seedlings of eggplants were transplanted singly to these pots. These pots were inoculated with freshly hatched five hundred 2nd stage juveniles (j_2) of *M. incognita* per 500g soil. The bioagents, either *Paecilomyces lilacinus* or *Trichoderma harzianum* were also inoculated simultaneously @ 0.5 g fungal mycelium per 500g soil. Freshly hatched third stage juveniles of EPN'S (*Steinernema* sp.) were also inoculated one week before the inoculation of *M. incognita* @ 5000 J_3 per 500 g soil. Carbofuran @0.25 g/500 g soil were also added followed by watering. The uninoculted and untreated pots served as control. To the one set of pots, freshly hatched 2nd stage juveniles of *M. incognita* were applied alone @ 500 J_2 *M. incognita* per 500 soil. Overall, there were 12 set of treatments besides control. These treatments are as follows.

1. Control (un-inoculated)

2. *Meloidogyne incognita*
3. *M. incognita* + Entomopathogenic nematodes (EPN'S) + Carbofuran
4. *M .incognita* + Carbofuran
5. *M .incognita* + EPN's + *Paecilomyces lilacinus*
6. *M. incognita* + EPN's + *Trichoderma harzianum*
7. *M. incognita* + *P.lilacinus*
8. *M. incognita* + EPN's + Neem cake
9. *M. incognita* + *T.harzianum*
10. *M. incognita* + EPN's + Soyabean cake
11. *M. incognita* + Neem cake
12. *M. incognita* + EPN's
13. *M. incognita* + Soyabean cake

Each set of treatment had five replicates.

The experiments were terminated after 60 days of transplantation. The soils from the roots were removed. The plant growth parameters like plant length, plant dry weight, number of flowers per plant, fruit weight per plant, nematode population of *M. incognita*, reproduction factor $R_F = \frac{P_F}{P_i}$, and number of galls per root system were recorded.

16. Statistical analysis:-

Data were stastically analyzed by one way analysis of variance (Anova) using SPSS 12.00 software(SPSS Inc., Chicago, IL, USA). C.D. was calculated at p=0.01 and p = 0.05 to test for significant differences.

17. Duncan Multiple Range analysis:

All the data was compared among treatment means by Duncans's Multiple Range Test (SAS Institute, 1999).

Chapter 4

RESULTS

CHAPTER 4

RESULTS

4.1. Studies on the frequency of occurrence of entomopathogenic nematodes in Aligarh district of U.P. (India):-

A Preliminary survey was conducted by collecting the soil samples from five different localities in and around Aligarh to isolate the entomopathogenic nematodes (EPN's). Eight late instar *Corcyra cephalonia* larvae were used for baiting in each collected soil samples over a period of 10 days. The results presented in table 1.1 clearly indicated that out of 195 soil samples collected from different localities, 19 samples showed the presence of EPN's. Among these 19 positive soil samples, only one sample collected from the Mathura road side showed the presence of *Heterorhabditis* sp., whereas, the remaining 18 positive samples showed the presence of *Steinernema* sp. Highest frequency of occurrence of EPN's (14.3%) was observed in the soil samples collected from Anoopshahar road followed by Mathura road (12.7%), A.M.U. Campus (8.9%) and Agra road (8.6%). However none of the soil samples collected from Ramghat road showed the presence of EPN's. The percentage mortality of larvae of *C. cephalonica* after 10 days of baiting caused by EPN's was recorded as 70.0%, 60.8%, 75.0% and 79.1% in the corresponding localities (Table 1.2).

4.2. Studies on the frequency of occurrence of entomopathogenic nematodes in two districts of Jammu and Kashmir (India):-

4.2.1. Pulwama District:-

A preliminary survey was conducted by collecting soil samples from eight different localities in and around Pulwama district of Kashmir (J & K) to isolate the entomopathognic nematodes (EPN's). Eight late instar *Corcyra cephalonica*

Table 1.1. Frequency of occurrence of EPN's in Aligarh district of U.P. (India).

Locality	No. of soil samples collected	No. of samples +ve for EPN's	Frequency of occurrence of EPN's	Genera identified
A.M.U. Campus	45	4	8.9	All samples showed *Steinernema* sp.
Anoop shahar Road	35	5	14.3	All samples showed *Steinernema* sp.
Mathura Road	55	7	12.7	6 samples showed *Steinernema* sp. 1 sample showed *Heterorhabditis* sp.
Agra Road	35	3	8.6	All samples showed *Steinernema* sp.
Ramghat Road	25	Nil	0	Nil
Total	195	19	9.74	18 samples showed *Steinernema* sp. 1 sample showed *Heterorhabditis* sp.
C.D. at *P*=0.05	–	0.47	1.08	–
C.D. at *P*=0.01	–	0.68	1.57	–

Table 1.2. Mortality of larvae of *Corcyra cephalonica* by EPN's after 10 days of baiting from each positive soil sample of Aligarh district (U.P.).

Locality	No. of +ve soil samples	Genera identified	No. of *C. cephalonica* larvae used for baiting	Mortality of larvae of *C. cephalonica* from each +ve soil sample	Average mortality of larvae of *C. cephalonica* from each locality	Percentage mortality of *C. cephalonica* larvae from each +ve sample
A.M.U. Campus	4	All *Steinernema* sp.	8 8 8 8	6 5 7 6	6	75.0
Anoop shahar Road	5	All *Steinernema* sp.	8 8 8 8 8	6 4 5 6 7	5.6	70.0
Mathura Road	7	6 *Steinernema* sp.	8 8 8 8 8 8	5 7 6 4 7 3	4.9	60.8
		1 *Heterorhabditis* sp.	8	2		
Agra Road	3	All *Steinernema* sp.	8 8 8	6 7 6	6.33	79.1
Ramghat Road	Nil	–	–	–	–	–
C.D. at *P*=0.05	0.47	–	–	0.483	–	–
C.D. at *P*=0.01	0.68	–	–	0.646	–	–

larvae were used for baiting in each collected soil samples over a period of 10 days. The results depicted in Table 2.1 clearly showed that out of 270 soil samples collected from different localities, 26 samples revealed the presence of EPN's. Among these 26 positive soil samples only two samples, one from each Chakoora-Pulwama road and Pampore showed the presence of *Heterorhabditis* sp., whereas the remaining 24 positive samples showed the presence of *Steinernema* sp. Highest frequency of occurrence of EPN's were observed in the soil collected from Kakapora (15.0%) followed by Pampore (14.3%), Chakoora-pulwama road and Awantipora (13.3 % each), Litter-Pulwama road (12.5 %) and Tral (2.9 %). However, none of the soil samples collected from Rajpora showed the presence of EPN's. The percentage mortality of larvae of *C. cephalonica* after 10 days of baiting caused by EPN's collected from the corresponding localities was recorded as 75.0 %, 60.0%, 67.5 %, 75.0%, 80.0 % and 87.5 %(Table 2.2).

4.2.2. Shopian District:-

A preliminary survey was carried out by collecting soil samples from seven different localities in and around Shopian district of Kashmir (J & K) to isolate the entomopathogenic nematodes (EPN's). Eight late instar *Corcyra cephalonica* larvae were used for baiting in each collected soil samples over a period of 10 days. The results presented in table 3.1 clearly indicated that out of 255 soil samples collected from different localities, only 17 samples showed the presence of EPN's. All the 17 positive samples showed the presence of *Steinernema* sp. However, none of the positive samples showed the presence of *Heterorhabditis* sp. Highest frequency of occurrence of EPN's was observed in the soil collected from Imamsahab (13.3%) followed by Zainapora (12.5 %), Chitragam (11.1 %) and Keller (8.6 %). However, none of the soil samples collected from Hurpora, Sedu and Mimander showed the presence of EPN's. The percentage mortality of larva of *C. Cephalonica* after 10 days of baiting caused by EPN's was recorded as 91.3 %, 75.0 %, 72.5 %, and 78.8 % in the corresponding localities(Table 3.2).

Table 2.1. Frequency of occurrence of EPN's in Pulwama district of Jammu & Kashmir (India).

Locality	No. of soil samples collected	No. of samples +ve for EPN's	Frequency of occurrence of EPN's	Genera identified
Rajpora	40	Nil	–	–
Tral	35	1	2.9	*Steinernema* sp.
Litter-Pulwama Road	40	5	12.5	All samples showed *Steinernema* sp.
Awantipora	30	4	13.3	All samples showed *Steinernema* sp.
Muran-Pulwama Road	25	2	8.0	All samples showed *Steinernema* sp.
Chakoora-Pulwama Road	45	6	13.3	5 samples showed *Steinernema* sp. 1 sample showed *Heterorhabditis* sp.
Pampore	35	5	14.3	4 samples showed *Steinernema* sp. 1 sample showed *Heterorhabditis* sp.
Kakapora	20	3	15.0	All *Steinernema* sp.
Total	270	26	9.63	–
C.D. at *P*=0.05	–	0.39	1.19	–
C.D. at *P*=0.01	–	0.55	1.65	–

Table 2.2. Mortality of larvae of *C. caphalonica* by EPN's after 10 days of baiting from each positive soil sample of Pulwama district (J&K).

Locality	No. of +ve soil samples	Genera identified	No. of *C. cephalonica* larvae used for baiting	Mortality of larvae of *C. cephalonica* from each +ve soil sample	Average mortality of larvae of *C. cephalonica* from each locality	Percentage mortality of *C. cephalonica* larvae from each +ve sample
Rajpora	Nil	–	–	–	–	–
Tral	1	*Steinernema* sp.	8	7	7	87.5
Litter-Pulwama Road	5	All *Steinernema* sp.	8 8 8 8 8	6 7 5 8 6	6.4	80.0
Awantipora	4	All *Steinernema* sp.	8 8 8 8	5 8 7 4	6.0	75.0
Muran-Pulwama Road	2	All *Steinernema* sp.	8 8	6 7	6.5	81.3
Chakoora-Pulwama Road	6	5 *Steinernema* sp.	8 8 8 8 8	6 5 6 4 6	5.4	67.5
		1 *Heterorhabditis*	8	5		
Pampore	5	4 *Steinernema* sp.	8 8 8 8	6 4 4 5	4.8	60.0
		1 *Heterorhabditis*	8	5		
Kakapora	3	All *Steinernema* sp.	8 8 8	7 5 6	6.0	75.0
C.D. at *P*=0.05	0.39	–	–	0.480	–	–
C.D. at *P*=0.01	0.55	–	–	0.638	–	–

Table 3.1. Frequency of occurrence of EPN's in Shopian district of Jammu & Kashmir (India).

Locality	No. of soil samples collected	No. of samples +ve for EPN's	Frequency of occurrence of EPN's	Genera identified
Hurpora	50	Nil	Nil	Nil
Sedu	35	Nil	Nil	Nil
Zainapora	40	5	12.5	All samples showed *Steinernema* sp.
Keller	35	3	8.6	All samples showed *Steinernema* sp.
Chitragam	45	5	11.1	All samples showed *Steinernema* sp.
Imamsahab	30	4	13.3	All samples showed *Steinernema* sp.
Mimander	20	Nil	Nil	Nil
Total	255	17	6.66	–
C.D. at *P*=0.05	3.87	–	0.94	–
C.D. at *P*=0.01	5.43	–	1.32	–

Table 3.2. Mortality of larvae of *C. caphalonica* by EPN's after 10 days of baiting from each positive soil sample of Shopian district of J&K.

Locality	No. of +ve soil samples	Genera identified	No. of *C. cephalonica* larvae used for baiting	Mortality of larvae of *C. cephalonica* from each +ve soil sample	Average mortality of larvae of *C. cephalonica* from each locality	Percentage mortality of *C. cephalonica* larvae from each +ve sample
Hurpora	Nil	Nil	Nil	Nil	Nil	Nil
Sedu	Nil	Nil	Nil	Nil	Nil	Nil
Zainapora	5	All *Steinernema* sp.	8 8 8 8 8	8 6 4 6 6	6.0	75.0
Keller	3	All *Steinernema* sp.	8 8 8	5 7 7	6.3	78.8
Chitragam	5	All *Steinernema* sp.	8 8 8 8 8	7 6 7 4 5	5.8	72.5
Imamsahab	4	All *Steinernema* sp.	8 8 8 8	8 7 8 6	7.3	91.3
Mimander	Nil	Nil	Nil	Nil	Nil	Nil
C.D. at *P*=0.05	0.36	–	–	0.511	–	–
C.D. at *P*=0.01	0.50	–	–	0.684	–	–

4.3. Effect of application of EPN's at different concentrations on the growth of eggplant seedlings:-

The data presented in Table 4.0 and figure 1.0 revealed that no significant variation in plant growth parameters (plant length, dry weight, number of flowers and weight of fruits) was recorded in the plants inoculated with different inoculums levels (50, 500, 1000, 2500, 5000, 10,000 and 20, 000 J_3/500 g soil) of EPN's viz., *Heterorhabditis* sp. and *Steinernema* sp. as compared to uninoculated plants

4.4. Management of *Meloidogyne incognita* infecting eggplant by using *Steinernema* sp. during concomitant inoculation:-

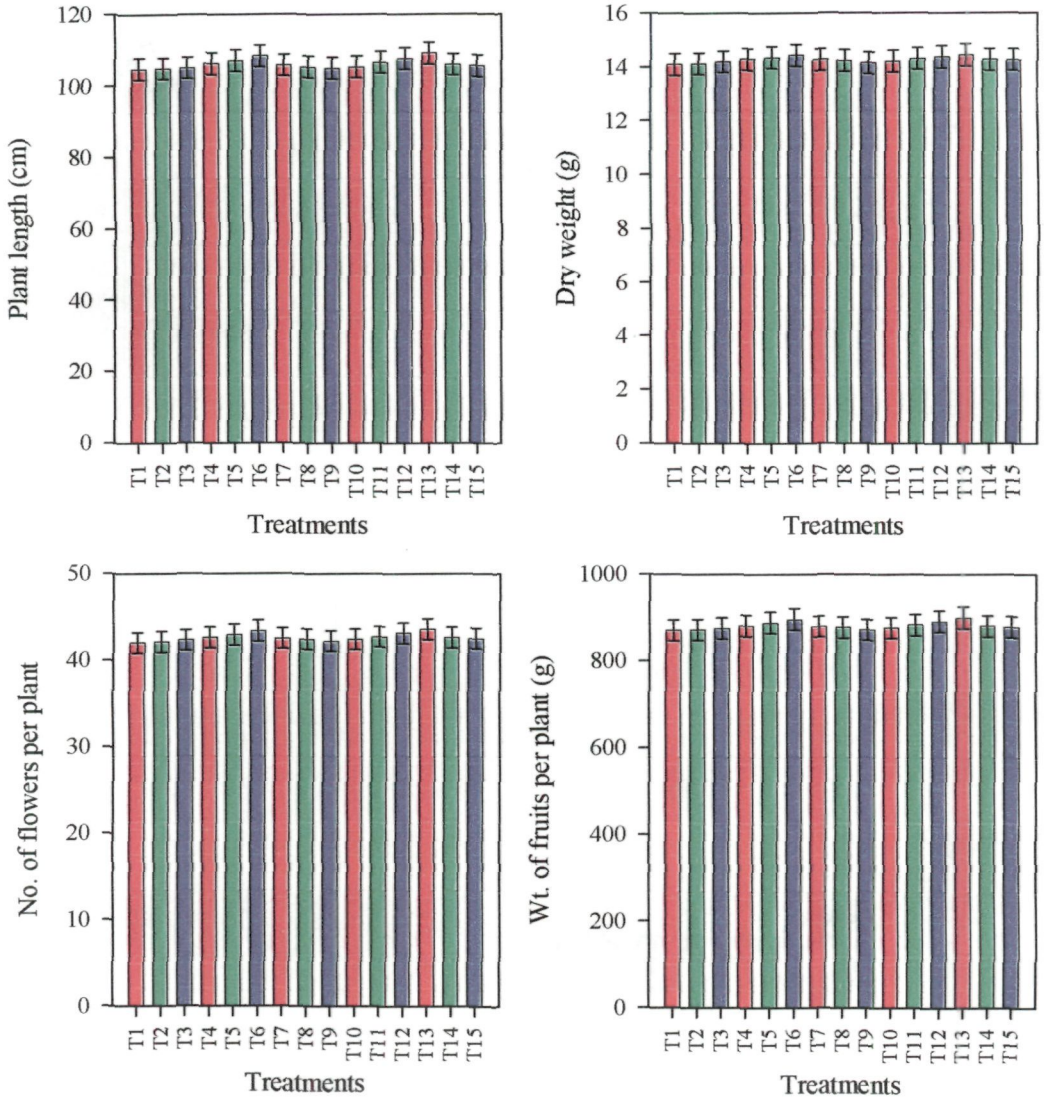
The data presented in Table 5.1 and figure 2.1 revealed that inoculation of eggplant seedlings with *M. incognita* caused significant reduction in plant growth parameters viz. plant length, dry weight, number of flowers and weight of fruits as compared to control plants. The percentage reduction caused by *M. incognita* in plant length, dry weight, number of flowers and weight of fruits was recorded as 37.50 %, 42.01 %, 49.42% and 43.18% respectively.

Moreover, the simultaneous inoculation of *M. incognita* and either of the inoculum levels (1000, 2500, 5000 and 10,000 J_3/500 g soil) of *Steinernema* sp. significantly reduced the damage caused by *M. incognita* in terms of plant growth parameters viz., plant length, dry weight, number of flowers and weight of fruits. The highest improvement in plant growth parameters viz., plant length, dry weight, number of flowers and weight of fruits was recorded in plants inoculated with 5000 J_3 of *Steinernema* sp. / 500g soil followed by 2500, 1000 and 10,000 J_3 / 500 g soil. In the corresponding treatments, the percentage reduction in length was recorded as 17.27%, 23.85%, 30.06% and 30.82% and dry weight as 19.85%, 26.86%, 33.80% and 35.83% and number of flowers as 16.40%, 30.25%, 38.98% and 39.95% and in weight of fruits was found to be as 21.02%, 28.98%, 35.80% and 36.93% as compared to control.

Table 4.0 Effect of application of EPN's at different concentrations on the growth of eggplant.

Treatments	Inoculum level (per 500g soil)	Plant growth (cm)				Dry weight (g)				No.of flowers/ plant	% increase over control	Weight of fruits/ plant	% increase over control
		Shoot	Root	Total	% increase over control	Shoot	Root	Total	% increase over control				
Control (uninoculated & untreated)	_	73.5	31.3	104.8	_	12.62	1.50	14.10	_	42.0	_	870	_
***Heterorhabditis* sp.**	50 J_3	73.5	31.4	104.9	+0.10	12.62	1.51	14.13	+0.21	42.1	+0.24	872	+0.23
	500 J_3	73.6	31.5	105.1	+0.29	12.68	1.52	14.20	+0.71	42.3	+0.71	875	+0.57
	1000 J_3	74.3	32.0	106.3	+1.43	12.74	1.55	14.29	+1.35	42.6	+1.43	881	+1.26
	2500 J_3	74.8	32.3	107.1	+2.19	12.77	1.58	14.35	+1.77	42.9	+2.14	888	+2.10
	5000 J_3	75.6	32.8	108.4	+3.44	12.81	1.62	14.43	+2.34	43.4	+3.33	896	+2.99
	10000 J_3	74.0	31.8	105.8	+0.95	12.72	1.54	14.26	+1.13	42.5	+1.19	879	+1.03
	20000 J_3	73.7	31.6	105.3	+0.48	12.70	1.53	14.23	+0.92	42.4	+0.95	877	+0.80
***Steinernema* sp.**	50 J_3	73.6	31.4	105.0	+0.19	12.64	1.51	14.15	+0.35	42.2	+0.48	872	+0.23
	500 J_3	73.7	31.6	105.3	+0.48	12.68	1.53	14.21	+0.78	42.4	+0.95	876	+0.69
	1000 J_3	74.5	32.1	106.6	+1.72	12.75	1.55	14.30	+1.42	42.7	+1.67	883	+1.49
	2500 J_3	75.0	32.6	107.6	+2.67	12.78	1.59	14.37	+1.91	43.1	+2.62	891	+2.41
	5000 J_3	75.9	33.2	109.1	+4.10	12.81	1.63	14.44	+2.41	43.6	+3.81	900	+3.45
	10000 J_3	74.2	31.8	106.0	+1.15	12.72	1.55	14.27	+1.20	42.6	+1.43	880	+1.15
	20000 J_3	73.9	31.7	105.6	+0.77	12.71	1.54	14.25	+1.06	42.5	+1.19	878	+0.92
C.D. at *P*=0.05	_	NS	NS	NS	_	NS	NS	NS	_	NS	-	NS	-
C.D. at *P*=0.01	_	NS	NS	NS	_	NS	NS	NS	_	NS	-	NS	-

Values are mean of five replicates
NS = non-significant



T1 = Control (uninoculated untreated), T2 = *Heterorhabditis* 50 J_3,
T3 = *Heterorhabditis* 500 J_3, T4 = *Heterorhabditis* 1000 J_3, T5 = *Heterorhabditis* 2500 J_3,
T6 = *Heterorhabditis* 5000 J_3, T7 = *Heterorhabditis* 10000 J_3, T8 = *Heterorhabditis* 20000 J_3,
T9 = *Steinernema* 50 J_3, T10 = *Steinernema* 500 J_3, T11 = *Steinernema* 1000 J_3,
T12 = *Steinernema* 2500 J_3, T13 = *Steinernema* 5000 J_3, T14 = *Steinernema* 10000 J_3,
T15 = *Steinernema* 20000 J_3

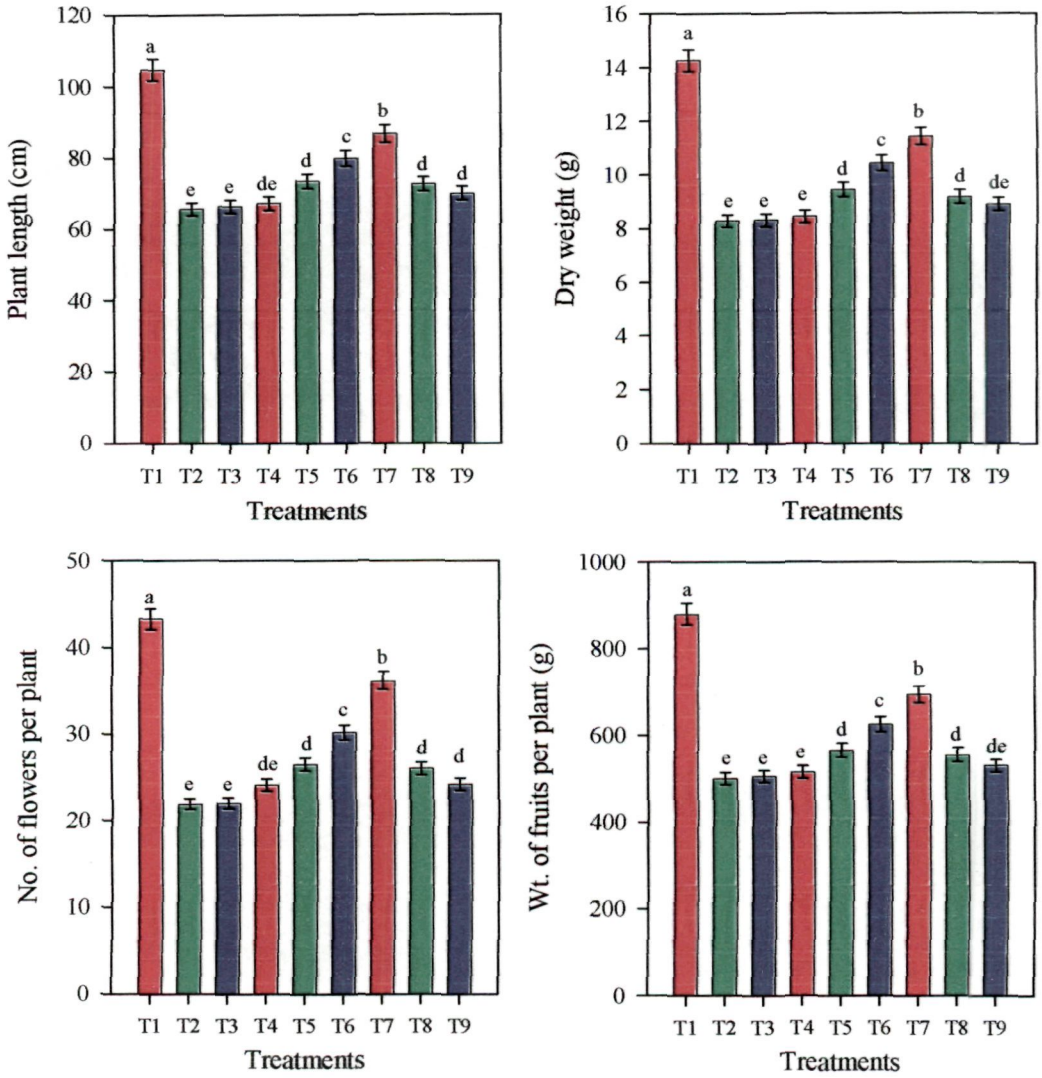
Bars with the same letters are non significant among the treatments according to Duncan's multiple range test ($P \leq 0.05$).

Fig. 1.0 Effect of EPN's at different concentrations on the growth of eggplants.

Table 5.1 Effect of different inoculum levels of *Steinernema* sp. during concomitant inoculation on the growth of eggplant infected with *Meloidogyne incognita*.

Treatments Inoculum levels/500g soil	Plant length (cm)				Dry weight (g)				No. of flowers per plant	% decrease from control	Wt. of fruits/ plant (g)	% decrease from control
	Shoot	Root	Total	% decrease from control	Shoot	Root	Total	% decrease from control				
Control (uninoculated untreated)	73.5	31.3	104.8	-	12.75	1.54	14.26	-	43.3	-	880	-
***M. incognita* (Mi)**	44.2**	21.3**	65.50**	-37.50	7.19**	1.08**	8.27**	-42.01	21.9**	-49.42	500**	-43.18
50J_3 EPN + Mi	44.8**	21.4**	66.20**	-29.96	7.22**	1.09**	8.31**	-41.73	22.0**	-49.19	505**	-42.61
500J_3 EPN + Mi	45.5**	21.6**	67.10**	-35.97	7.30**	1.13**	8.43**	-40.88	24.1**	-46.34	515**	-41.48
1000J_3 EPN + Mi	50.6**	22.7**	73.40**	-30.06	8.20**	1.24**	9.44**	-33.80	26.5**	-38.80	565**	-35.80
2500J_3 EPN + Mi	54.9**	24.9**	79.80**	-23.85	9.10**	1.33**	10.43**	-26.86	30.2**	-30.25	625**	-28.98
5000J_3 EPN + Mi	60.2**	26.5**	86.70**	-17.27	10.00**	1.43*	11.43**	-19.85	36.2**	-16.40	695**	-21.02
10000J_3 EPN + Mi	50.2**	22.3**	72.50**	-30.82	8.08**	1.07**	9.15**	-35.83	26.0**	-39.95	555**	-36.93
20000J_3 EPN + Mi	48.0**	21.9**	69.90**	-33.30	7.75**	1.14**	8.89**	-37.66	24.2**	-44.11	530**	-39.77
C.D. at *P*=0.05	-	-	6.27	-	-	-	0.817	-	2.35	-	49.48	-
C.D. at *P*=0.01	-	-	8.64	-	-	-	1.126	-	3.24	-	68.18	-

Values are mean of five replicates
* significant over control at 5%;
** significant over control at 5% and 1%



T1 = Control (uninoculated untreated), T2 = *M. incognita* (Mi), T3 = 50J_3 EPN + Mi
T4 = 500J_3 EPN + Mi, T5 = 1000J_3 EPN + Mi, T6 = 2500J_3 EPN + Mi,
T7 = 5000J_3 EPN + Mi, T8 = 10000J_3 EPN + Mi, T9 = 20000J_3 EPN + Mi

Bars with the same letters are non significant among the treatments according to Duncan's multiple range test ($P \leq 0.05$).

Fig. 2.1 Effect of different inoculum levels of *Steinernema* sp. during concomitant inoculation on the growth of eggplant infected with *Meloidogyne incognita*.

The data presented in table 5.2 and figure 2.2 revealed that multiplication of root-knot nematode *M. incognita* was found to be higher in case of *M. incognita* inoculated eggplants. The reproduction factor and number of galls / root system was found to be as 14.72 and 80, respectively.

However, on the other hand, the inoculation of eggplants with *Steinernema* sp. at moderate inoculum levels i.e. 1000, 2500, 5000 and 10,000 significantly reduced the multiplication of *M. incognita* and number of galls / root system. The highest reduction in the reproduction factor (Rf) and number of galls of root-knot nematode was recorded in the plants treated with 5000 J_3 *Steinernema* sp. / 500 g soil followed by 2500, 1000 and 10,000 J_3 / 500 g soil. In the corresponding treatments the reproduction factor (Rf) was recorded as 7.50, 10.20, 12.90 and 13.40 and number of galls / root system was found to be as 40, 58, 72 and 76.Moreover, the inoculation of *Steinernema* sp. at lower (50,500) and higher (20,000) inoculums levels neither significantly improved growth parameters and nor reduce the reproduction factor and number of galls per root system.

4.5. Management of *Meloidogyne incognita* infecting eggplant by sequential inoculation of *Steinernema* sp., one weak prior to *M. incognita*:-

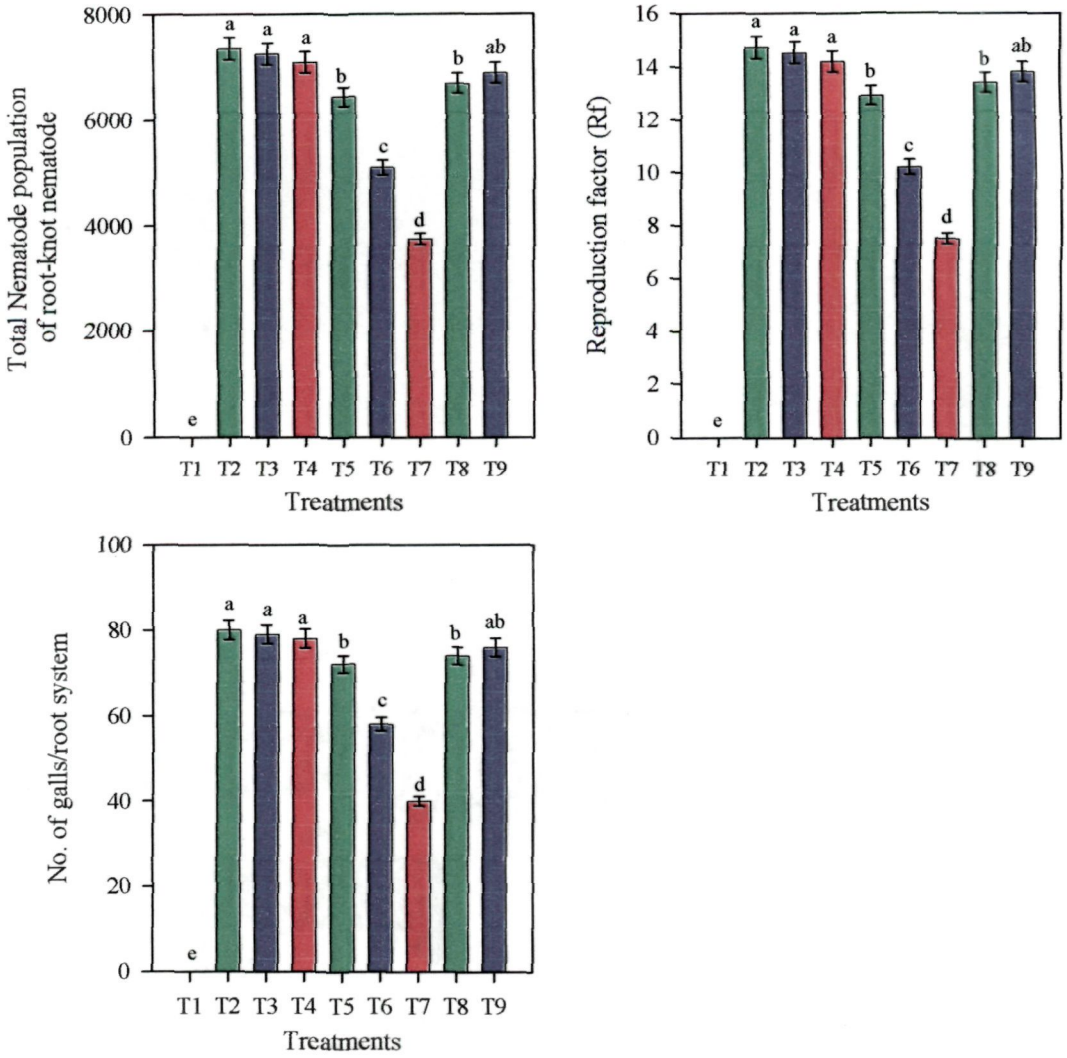
The data presented in Table 6.1 and figure 3.1 revealed that inoculation of eggplant seedlings with *M. incognita* caused significant reduction in plant growth parameters viz., plant length, dry weight, number of flowers and weight of fruits as compared to the control plants. The percentage reduction caused by *M. incognita* in plant length, dry weight, number of flowers and weight of fruits was recorded as 37.50% 42.01%, 49.42% and 43.18% respectively.

However, the sequential inoculation of *Steinernema* sp. with either of the inoculums levels (1000,2500,5000,10,000 and 20,000) one week before the inoculation of *M. incognita* in eggplants significantly reduced the damage in terms of plant growth parameters viz., plant length, dry weight, number of flowers and

Table 5.2 Effect of different inoculums levels of *Steinernema* sp. during concomitant inoculation on the multiplication of *Meloidogyne incognita* infecting eggplant.

Treatments Inoculum levels/500g soil	Nematode population of *M. incognita* / 500g soil				No. of galls/ root system
	Females	Larvae	Total	R.f.	
Control (uninoculated untreated)	–	–	–	–	–
***M. incognita* (Mi)**	230	7130	7,360**	14.72**	80**
50J_3 EPN + Mi	225	7035	7,260**	14.52**	79**
500J_3 EPN + Mi	205	6895	7,100**	14.20**	77**
1000J_3 EPN + Mi	195	6255	6,450**	12.90**	72**
2500J_3 EPN + Mi	170	4930	5,100**	10.20**	58**
5000J_3 EPN + Mi	145	3605	3,750**	7.50**	40**
10000J_3 EPN + Mi	200	6500	6,700**	13.40**	74**
20000J_3 EPN + Mi	205	6695	6,900**	13.80**	76**
C.D. at *P*=0.05	–	–	488.54	0.98	5.34
C.D. at *P*=0.01	–	–	673.12	1.35	7.36

Values are mean of five replicates
* significant over control at 5%;
** significant over control at 5% and 1%



T1 = Control (uninoculated untreated), T2 = *M. incognita* (Mi), T3 = $50J_3$ EPN + Mi
T4 = $500J_3$ EPN + Mi, T5 = $1000J_3$ EPN + Mi, T6 = $2500J_3$ EPN + Mi,
T7 = $5000J_3$ EPN + Mi, T8 = $10000J_3$ EPN + Mi, T9 = $20000J_3$ EPN + Mi

Bars with the same letters are non significant among the treatments according to Duncan's multiple range test ($P \leq 0.05$).

Fig. 2.2 Effect of different inoculum levels of *Steinernema* sp. during concomitant inoculation on the multiplication of *Meloidogyne incognita* infecting eggplant.

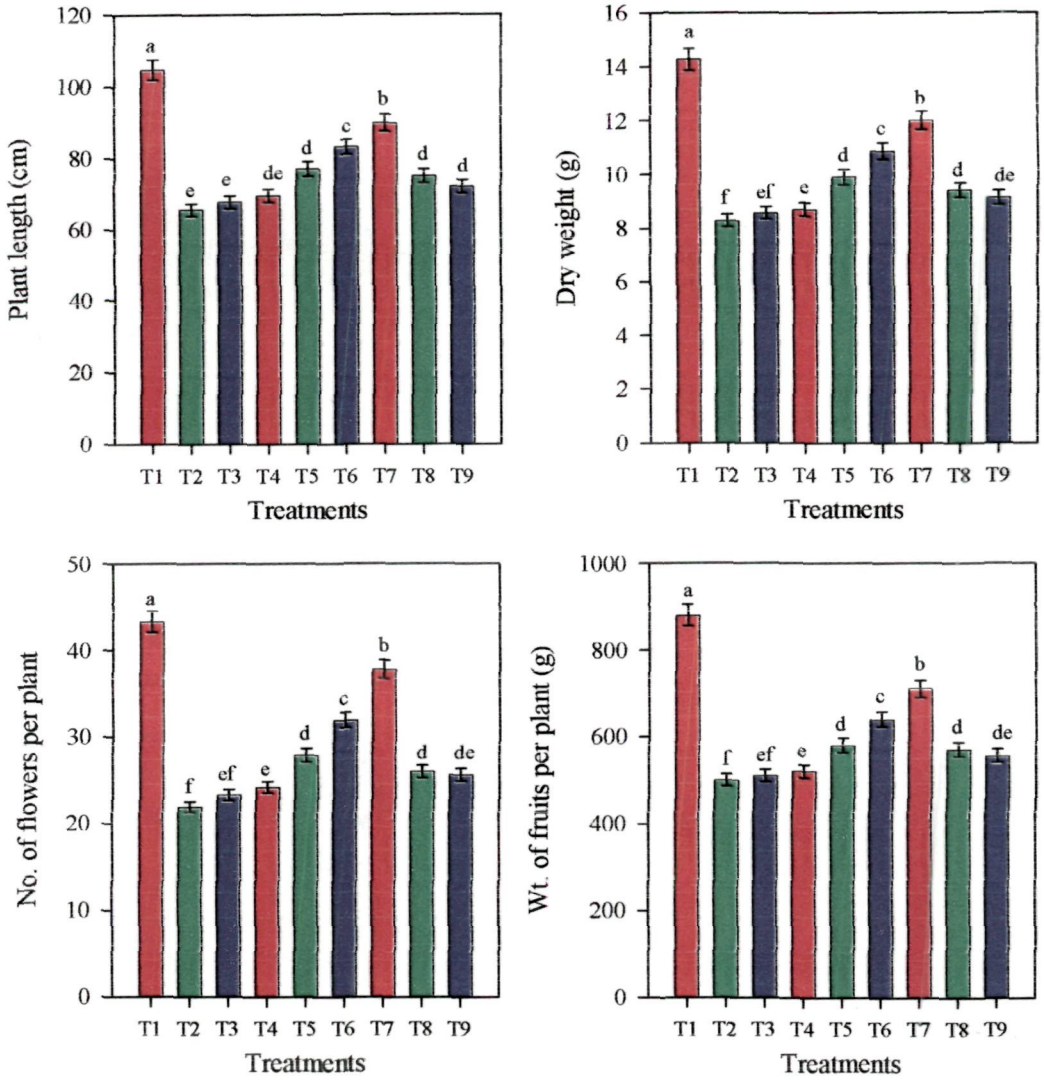
weight of fruits caused by *M. incognita*. The highest improvement in plant growth parameters viz., plant length, dry weight, number of flowers and weight of fruits was recorded in the plants treated with 5000 J_3 *Steinernema* sp. / 500 g soil followed by 2500, 1000, 10,000 and 20,000 J_3 *Steinernema* sp. / 500 g soil. The significant improvement in the plant growth parameters was noticed in all these treatments as compared to plants inoculated with *M. incognita* alone. In the corresponding treatments the percentage reduction in length was recorded as 11.55%, 18.99%, 25.57%, 27.96% and 30.34% and dry weight 12.69%, 20.83%, 28.61%, 33.03% and 34.43% and number of flowers as 11.09%, 24.94%, 35.33%, 39.26% and 39.95% and weight of fruits found to be as 16.48%, 25.57%, 32.95%, 33.52% and 36.93% in comparison to control.

The data presented in table 6.2 and figure3.2 revealed that root-knot nematode, *M. incognita* multiplication and number of galls per root system was found to be significantly higher in case of *M. incognita* inoculated egg plants. However, the inoculation of *Steinernema* sp. at different inoculum levels (1000, 2500, 5000, 10,000 and 20,000) one week before the inoculation of *M. incognita* in eggplants significantly reduced the *M. incognita* multiplication and number of galls / root system. The highest reduction in the reproduction factor (Rf) and number of galls of root-knot nematode was recorded in the plants treated with 5000 J_3 *Steinernema* sp. /500g soil followed by 2500, 1000, 10,000 and 20,000 J_3 / 500 g soil. In the corresponding treatments the reproduction factor was recorded as 4.00, 8.40, 12.60, 13.10 and 13.40 and number of galls/ root system was found to be as 30, 50, 70, 72 and 73.However on the other hand, the lower inoculum (50,500) of *Steinernema* sp. applied one week before the inoculation of *M. incognita* didn't significantly improve the eggplant growth parameters and reduce the reproduction factor and number of galls / root system as compared to the plants individually inoculated with *M. incognita*.

Table 6.1. Effect of different inoculums levels of *Steinernema* sp. applied during sequential inoculation, one week prior to *Meloidogyne incognita* on the growth of eggplant.

Treatments Inoculum levels/500g soil	Plant length (cm)				Dry weight (g)				No. of flowers per plant	% decrease from control	Wt. of fruits/ plant (g)	% decrease from control
	Shoot	Root	Total	% decrease from control	Shoot	Root	Total	% decrease from control				
Control (uninoculated untreated)	73.5	31.3	104.80	-	12.75	1.54	14.26	-	43.3	-	880	-
***M. incognita* (Mi)**	44.2**	21.3**	65.50**	-37.50	7.19**	1.08**	8.27**	-42.01	21.9**	-49.42	500**	-43.18
50J_3 EPN + Mi	45.8**	22.4**	68.20**	-34.92	7.50**	1.13**	8.63**	-39.48	23.6**	-45.50	515**	-41.48
500J_3 EPN + Mi	47.9**	23.6**	71.50**	-31.77	7.62**	1.18**	8.80**	-38.29	24.3**	-43.88	525**	-40.34
1000J_3 EPN + Mi	54.0**	24.0**	78.00**	-25.57	9.14**	1.04**	10.18**	-28.61	28.0**	-35.33	590**	-32.95
2500J_3 EPN + Mi	58.5**	26.4**	84.90**	-18.99	9.92**	1.37**	11.29**	-20.83	32.5**	-24.94	655**	-25.57
5000J_3 EPN + Mi	64.2**	28.5*	92.70**	-11.55	11.00**	1.45ns	12.45**	-12.69	38.5**	-11.09	735**	-16.48
10000J_3 EPN + Mi	51.5**	24.0**	75.50**	-27.96	8.35**	1.20**	9.55**	-33.03	26.3**	-39.26	575**	-34.66
20000J_3 EPN + Mi	49.2**	23.8**	73.00**	-30.34	8.00**	1.19**	9.35**	-34.43	26.0**	-39.95	555**	-36.93
C.D. at *P*=0.05	–	–	6.50	–	–	–	0.850	–	2.43	–	50.90	–
C.D. at *P*=0.01	–	–	8.96	–	–	–	1.171	–	3.41	–	70.13	–

Values are mean of five replicates
* significant over control at 5%:
** significant over control at 5% and 1%



T1 = Control (uninoculated untreated), T2 = *M. incognita* (Mi), T3 = $50J_3$ EPN + Mi
T4 = $500J_3$ EPN + Mi, T5 = $1000J_3$ EPN + Mi, T6 = $2500J_3$ EPN + Mi,
T7 = $5000J_3$ EPN + Mi, T8 = $10000J_3$ EPN + Mi, T9 = $20000J_3$ EPN + Mi

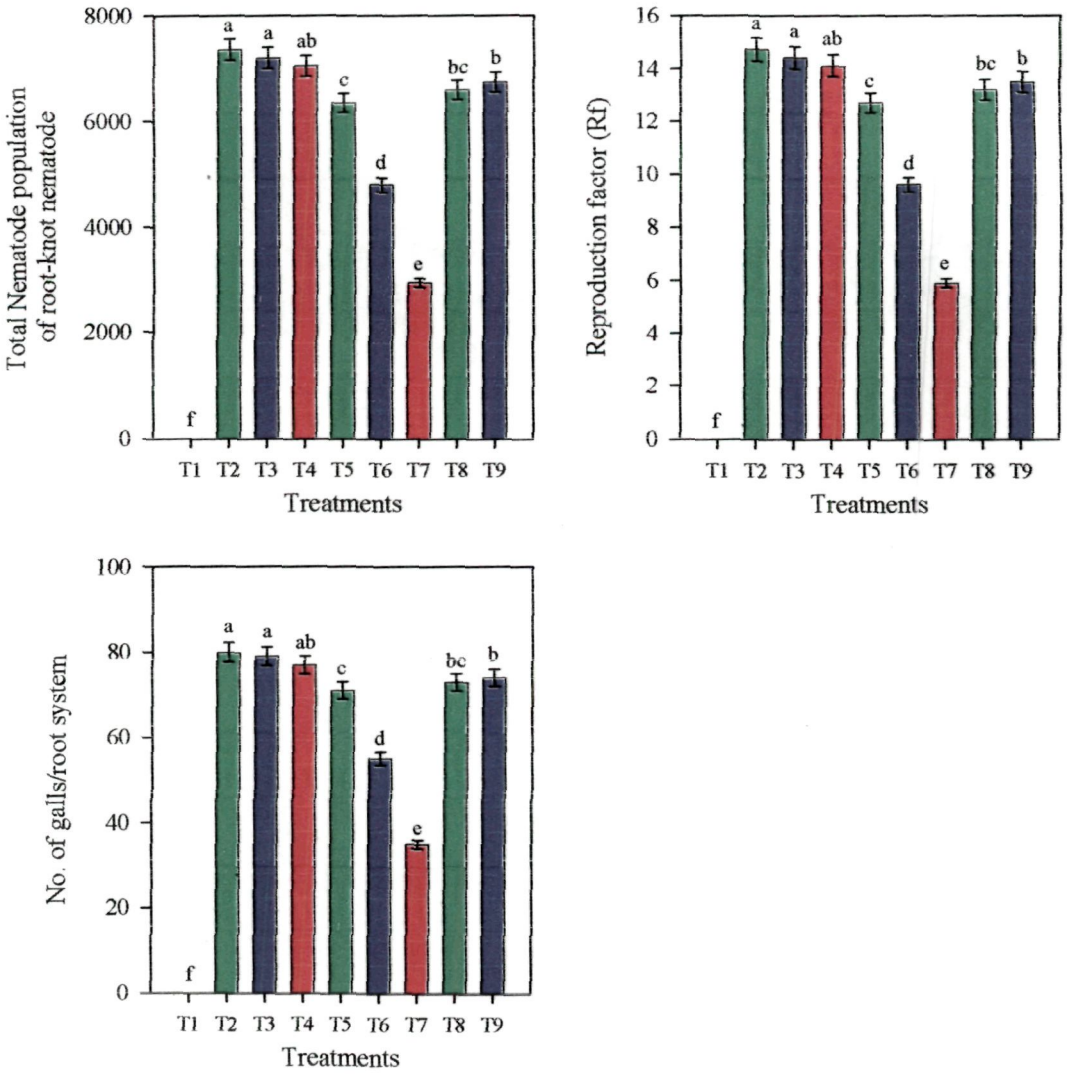
Bars with the same letters are non significant among the treatments according to Duncan's multiple range test ($P \leq 0.05$).

Fig. 3.1 Effect of different inoculums levels of *Steimernema* sp. applied during sequential inoculation, one week prior *M. incognita* on the growth of eggplant.

Table 6.2 Effect of different inoculums levels of *Steinernema* sp. applied during sequential inoculation, one week prior to *Meloidogyne incognita* on the multiplication of *M. incognita* infecting eggplant.

Treatments Inoculum levels/500g soil	Nematode population of *M. incognita* / 500g soil				No. of galls/ root system
	Females	Larvae	Total	R.f.	
Control (uninoculated untreated)	–	–	–	–	–
***M. incognita* (Mi)**	230	7130	7,360**	14.72**	80**
50J_3 EPN + Mi	210	6940	7,150**	14.30**	77**
500J_3 EPN + Mi	190	6810	7,000**	14.00**	76**
1000J_3 EPN + Mi	170	6130	6,300**	12.60**	70**
2500J_3 EPN + Mi	145	4055	4,200**	8.40**	50**
5000J_3 EPN + Mi	115	5240	2,000**	4.00**	30**
10000J_3 EPN + Mi	175	6375	6,550**	13.10**	72**
20000J_3 EPN + Mi	180	6520	6,700**	13.40**	73**
C.D. at *P*=0.05	–	–	460.08	0.95	4.90
C.D. at *P*=0.01	–	–	633.92	1.30	6.75

Values are mean of five replicates
* significant over control at 5%:
** significant over control at 5% and 1%



T1 = Control (uninoculated untreated), T2 = *M. incognita* (Mi), T3 = $50J_3$ EPN + Mi
T4 = $500J_3$ EPN + Mi, T5 = $1000J_3$ EPN + Mi, T6 = $2500J_3$ EPN + Mi,
T7 = $5000J_3$ EPN + Mi, T8 = $10000J_3$ EPN + Mi, T9 = $20000J_3$ EPN + Mi

Bars with the same letters are non significant among the treatments according to Duncan's multiple range test ($P \leq 0.05$).

Fig. 3.2 Effect of different inoculum levels of *Steinernema* sp. applied during sequential inoculation, one week prior *M. incognita* on the multiplication of *M. incognita* infecting eggplant.

4.6. Management of *Meloidogyne incognita* infecting eggplant by the sequential inoculation of *Steinernema* sp., one week after *M. incognita*:-

The data presented in table 7.1 and figure 4.1 showed that inoculation of eggplant seedlings with *M. incognita* caused significant reduction in plant growth parameters such as plant length, dry weight, number of flowers and weight of fruits as compared to un-inoculated and untreated plants. The percentage reduction caused by *M. incognita* in plant length , dry weight , number of flowers and weight of fruits was recorded as 37.50%,42.01%,49.42% and 43.18%,respectively, as compared to control.

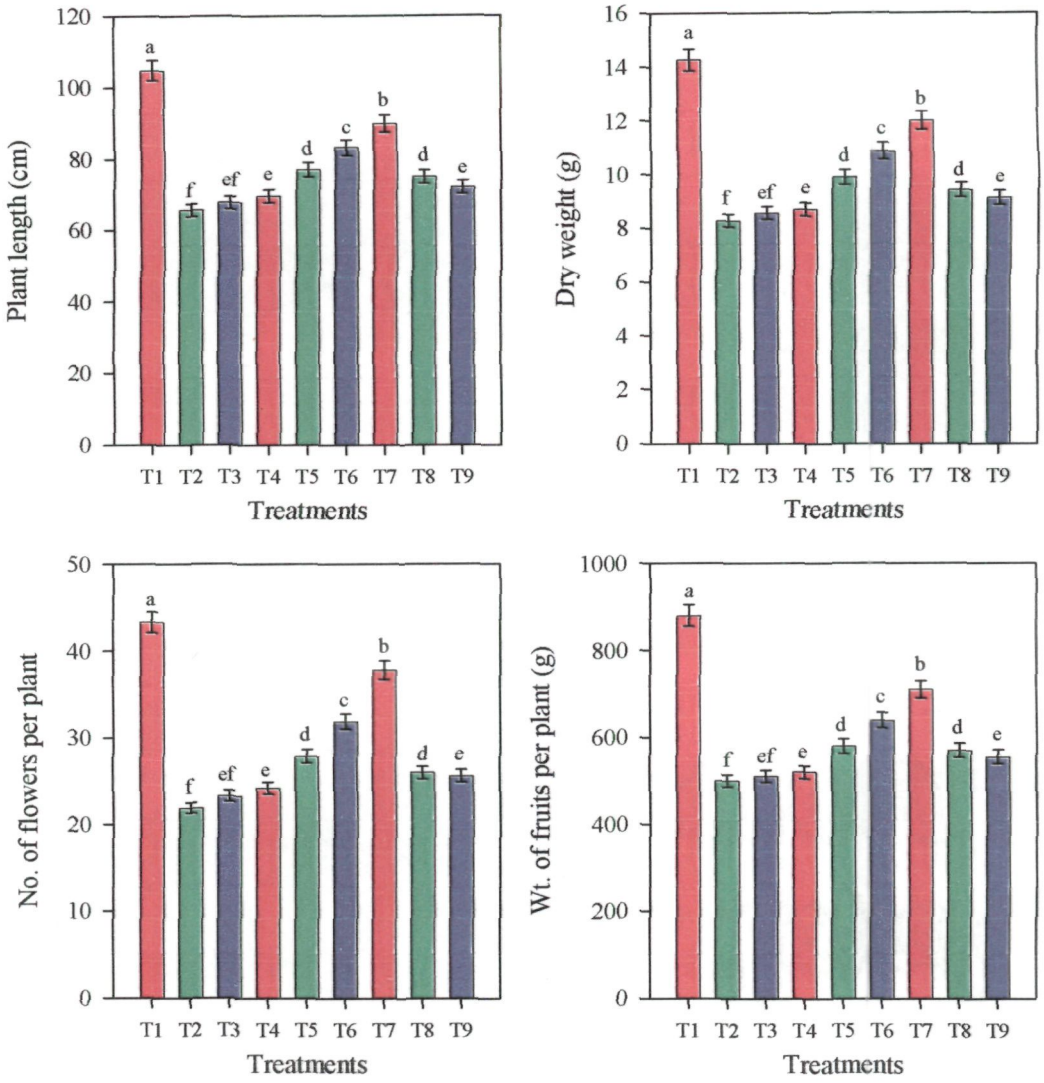
However, the sequential inoculation of *Steinernema* sp. with either of the inoculum levels (1000,2500,5000,10,000 and 20,000) one week after the inoculation of *M. incognita* in eggplants significantly reduced the damage caused by *M.incognita* in terms of plant growth parameters viz., plant length, dry weight, number of flowers and weight of fruits. The highest improvement in plant growth parameters viz., plant length, dry weight, number of flowers and weight of fruits was recorded in the plants treated with 5000 J_3 *Steinernema* sp. / 500 g soil followed by 2500, 1000, 10,000 and 20,000 J_3 *Steinernema* sp. / 500g soil. The significant improvement in the plant growth parameters was noticed in all these treatments as compared to plants inoculated with *M. incognita* alone. In the corresponding treatments the percentage reduction in length was recorded as 14.31%, 20.71%, 26.53%, 28.53% and 31.30% and dry weight as 15.85%, 23.84%, 30.58%, 34.08% and 36.04% and number of flowers as 12.70%, 26.33%, 35.57%, 39.95% and 40.88% and weight of fruits as 19.32%, 27.27%, 34.09%, 35.22% and 36.93% in comparison to control.

The data presented in table 7.2 and figure 4.2 revealed that root-knot nematode multiplication and number of gall/root system was found to be significantly higher in case of eggplant seedlings inoculated with *M. incognita.*

Table 7.1 Effect of different inoculum levels of *Steinernema* sp. applied during sequential inoculation, one week after *Meloidogyne incognita* on the growth of eggplant.

Treatments Inoculum levels/500g soil	Plant length (cm)				Dry weight (g)				No. of flowers per plant	% decrease from control	Wt. of fruits/ plant (g)	% decrease from control
	Shoot	Root	Total	% decrease from control	Shoot	Root	Total	% decrease from control				
Control (uninoculated untreated)	73.50	31.30	104.80	-	12.75	1.54	14.26	-	43.30	-	880.00	-
***M. incognita* (Mi)**	44.20**	21.30**	65.50**	-37.50	7.19**	1.08**	8.27**	-42.01	21.90**	-49.42	500.00**	-43.18
50J_3 EPN + Mi	45.50**	22.20**	67.70**	-35.40	7.45**	1.11**	8.56**	-39.97	23.30**	-46.19	510.00**	-42.04
500J_3 EPN + Mi	46.30**	23.20**	69.50**	-33.68	7.55**	1.14**	8.69**	-39.06	24.20**	-44.11	520.00**	-40.91
1000J_3 EPN + Mi	52.90**	24.10**	77.00**	-26.53	8.80**	1.20**	9.90**	-30.58	27.90**	-35.57	580.00**	-34.09
2500J_3 EPN + Mi	57.30**	25.80**	83.10**	-20.71	9.50**	1.36**	10.86**	-23.84	31.90**	-26.33	640.00**	-27.27
5000J_3 EPN + Mi	62.30**	27.50**	89.80**	-14.31	10.60**	1.40*	12.00**	-15.85	37.80**	-12.70	710.00**	-19.32
10000J_3 EPN + Mi	51.10**	23.80**	74.90**	-28.53	8.30**	1.10**	9.40**	-34.08	26.00**	-39.95	570.00**	-35.22
20000J_3 EPN + Mi	49.00**	23.00**	72.00**	-31.30	8.10**	1.02**	9.12**	-36.04	25.60**	-40.88	555.00**	-36.93
C.D. at *P*=0.05	–	–	6.00	–	–	–	0.84	–	2.40	–	50.50	–
C.D. at *P*=0.01	–	–	8.27	–	–	–	1.16	–	3.30	–	69.25	–

Values are mean of five replicates
* significant over control at 5%:
** significant over control at 5% and 1%



T1 = Control (uninoculated untreated), T2 = *M. incognita* (Mi), T3 = 50J_3 EPN + Mi
T4 = 500J_3 EPN + Mi, T5 = 1000J_3 EPN + Mi, T6 = 2500J_3 EPN + Mi,
T7 = 5000J_3 EPN + Mi, T8 = 10000J_3 EPN + Mi, T9 = 20000J_3 EPN + Mi

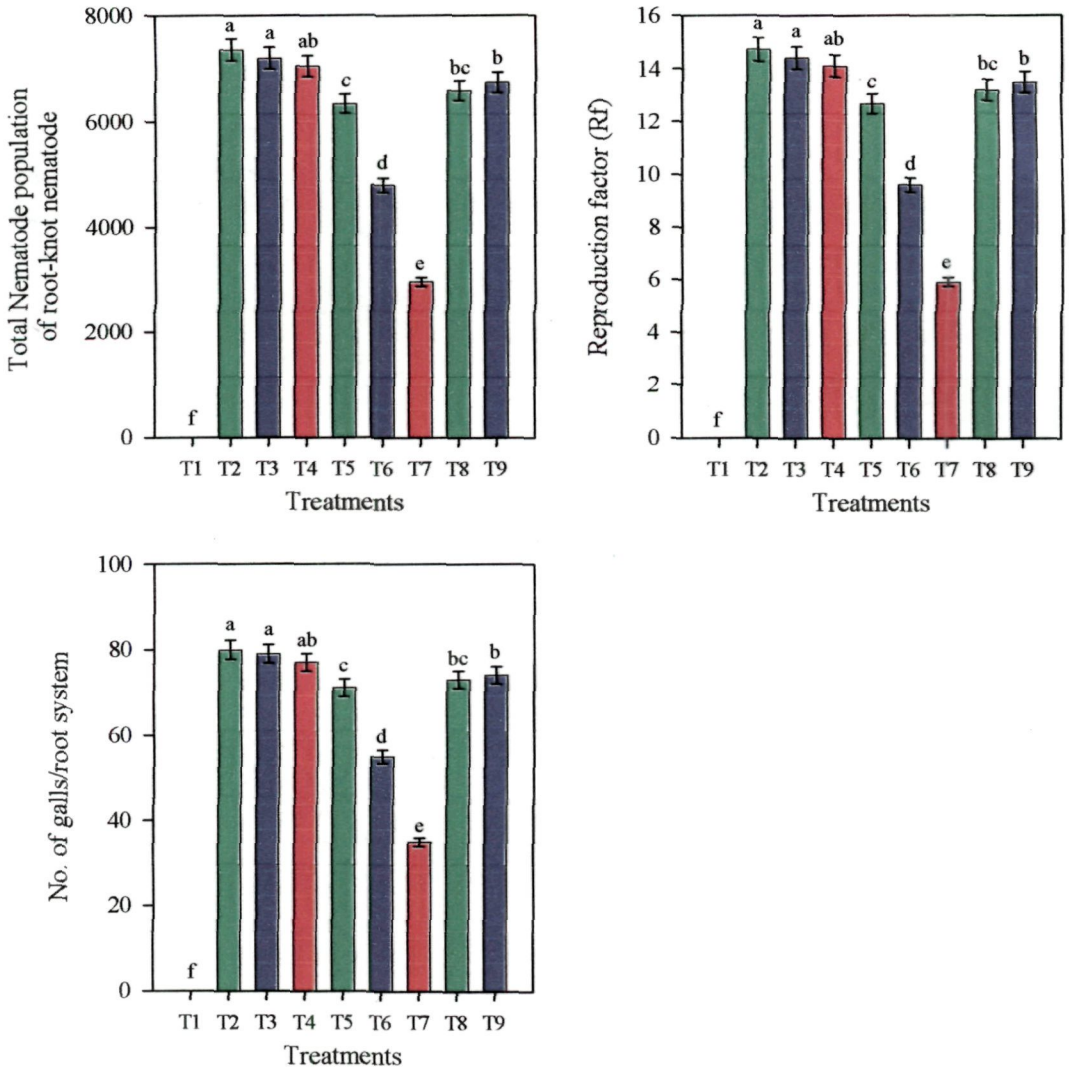
Bars with the same letters are non significant among the treatments according to Duncan's multiple range test ($P \leq 0.05$).

Fig. 4.1 Effect of different inoculum levels of *Steinernema* sp. applied during sequential inoculation, one week after *M. incognita* on the growth of eggplant.

Table 7.2 Effect of different inoculums levels of *Steinernema* sp. applied during sequential inoculation, one week after *M. incognita* on the multiplication of *Meloidogyne incognita* infecting eggplant.

Treatments Inoculum levels/500g soil	Nematode population of *M. incognita* / 500g soil				No. of galls/ root system
	Females/root system	Larvae/500g soil	Total	R.f. = Pf/Pi	
Control (uninoculated untreated)	–	–	–	–	–
M. incognita (Mi)	230	7130	7,360.00**	14.72**	80.00**
$50J_3$ EPN + Mi	220	6980	7,200.00**	14.40**	79.00**
$500J_3$ EPN + Mi	200	6850	7,050.00**	14.10**	78.00**
$1000J_3$ EPN + Mi	180	6170	6,350.00**	12.70**	71.00**
$2500J_3$ EPN + Mi	155	4645	4,800.00**	9.60**	55.00**
$5000J_3$ EPN + Mi	130	2820	2,950.00**	5.90**	35.00**
$10000J_3$ EPN + Mi	185	6415	6,600.00**	13.20**	73.00**
$20000J_3$ EPN + Mi	190	6560	6,750.00**	13.50**	74.00**
C.D. at *P*=0.05	–	–	470.08	0.98	5.00
C.D. at *P*=0.01	–	–	647.69	1.35	7.07

Values are mean of five replicates
* significant over control at 5%;
** significant over control at 5% and 1%



T1 = Control (uninoculated untreated), T2 = *M. incognita* (Mi), T3 = 50J_3 EPN + Mi
T4 = 500J_3 EPN + Mi, T5 = 1000J_3 EPN + Mi, T6 = 2500J_3 EPN + Mi,
T7 = 5000J_3 EPN + Mi, T8 = 10000J_3 EPN + Mi, T9 = 20000J_3 EPN + Mi

Bars with the same letters are non significant among the treatments according to Duncan's multiple range test ($P \leq 0.05$).

Fig. 4.2 Effect of different inoculums levels of *Steinernema* sp. applied during sequential inoculation, one week after *Meloidogyne incognita* on the multiplication *M. incognita* infecting eggplant.

The reproduction factor and number of galls per root system was found to be as 14.72 and 80 respectively.

However, the inoculation of *Steinernema* sp. at different inoculum levels (1000, 2500, 5000, 10,000 and 20,000) one week after the inoculation of *M. incognita* in eggplants significantly reduced the *M. incognita* multiplication and root galling. The highest reduction in the reproduction factor and number of galls per root system was reported in the plants treated with 5000 J_3 *Steinernema* sp. / 500g soil followed by 2500, 1000, 10,000 and 20,000 J_3 *Steinernema* sp./500g soil. In the corresponding treatments the reproduction factor was found to be as 5.90, 9.60, 12.70, 13.20 and 13.50 and the number of galls per root system as 35, 55, 71, 73 and 74.However on the other hand, the lower inoculum (50,500) of *Steinernema* sp. one week after the inoculation of *M. incognita* didn't significantly improve the eggplant growth parameters and reduce the reproduction factor and number of galls per root system as compared to the plants individually inoculated with *M. incognita.*

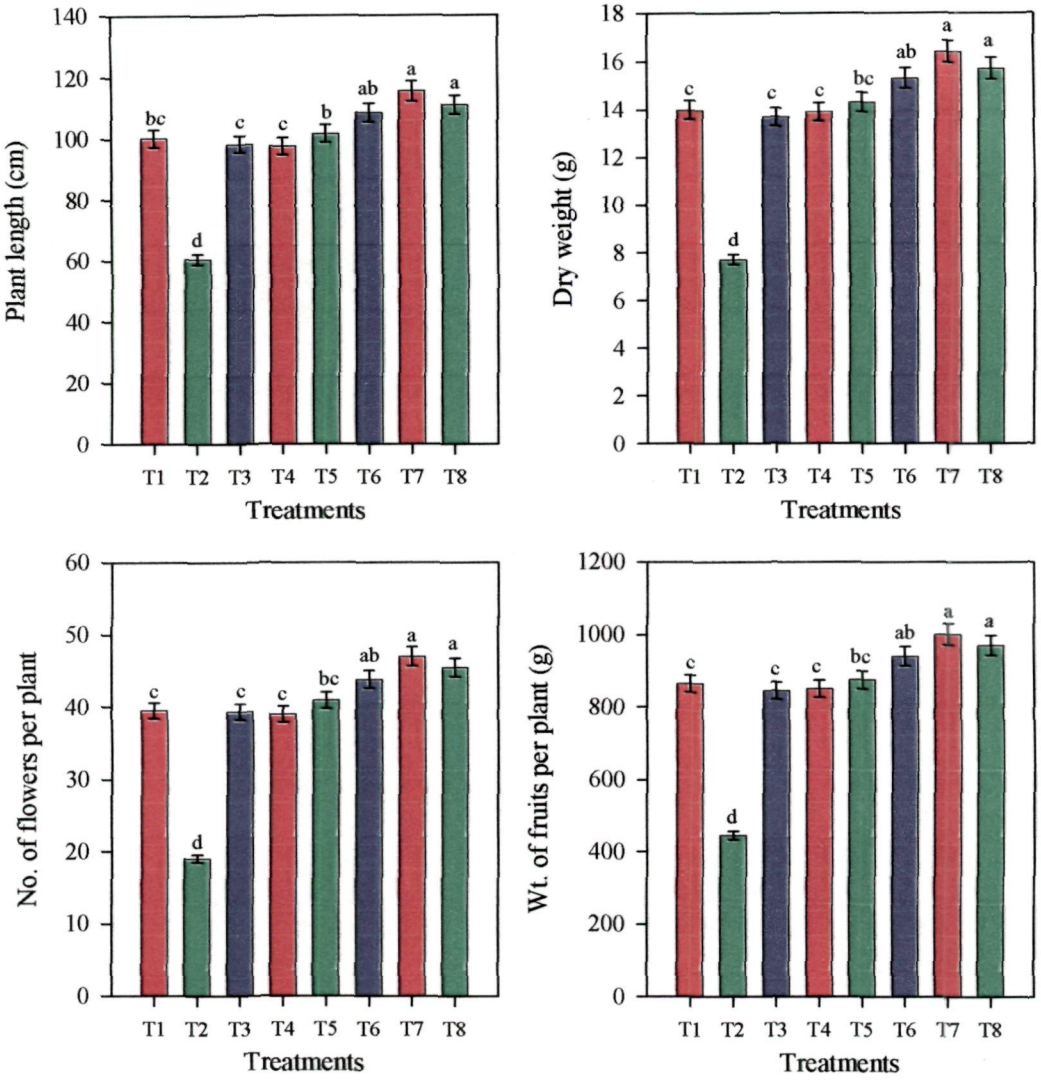
4.7. Effect of biocontrol agents, oil-cakes and nematicide-carbofuran on the growth of eggplant:-

The data presented in Table 8.0 and figure 5.0 revealed that the application of *Trichoderma harzianum*, Neem cake and Soyabean cake into the soil significantly improved the plant growth parameters such as plant length, dry weight, number of flowers and weight of fruits as compared to the plants grown in untreated soil (control). In the corresponding treatments the percentage improvement in plant length was recorded as 8.28%, 15.27% and 10.78% and dry weight as 9.29%, 17.14% and 12.14% and number of flowers as 10.89%, 18.98% and 15.19% and weight of fruits was recorded as 8.67%, 15.61% and 12.14% with respect to control (un-treated) plants. However, on the other hand, the application of carbofuran, *Paecilomyces lilacinus* and EPN's (*Steinernema* sp.)into the soil didn't significantly improved the plant growth parameters viz., plant length, dry

Table 8.0 Effect of treatment of biocontrol agents, oil cakes and carbofuran on the growth of eggplant.

Treatments	Plant length (cm)				Dry weight (g)				No. of flowers per plant	(% increase (+) or decrease (-) over control)	Wt. of fruits/ plant (g)	(% increase (+) or decrease (-) over control)
	Shoot	Root	Total	% increase (+) or decrease (-) over control	Shoot	Root	Total	% increase (+) or decrease (-) over control				
Control (uninoculated untreated)	70.30	29.90	100.20	-	12.49	1.51	14.00	-	39.50	-	865	-
Meloidogyne incognita	43.00	17.50	60.50**	-39.62	6.90	0.80	7.70**	-45.00	19.00**	-51.90	445**	-48.55
Carbofuran	69.30	29.00	98.30ns	-1.90	12.05	1.45	13.70ns	-2.14	39.30ns	-0.51	845ns	-3.47
Paecilomyces lilacinus	68.90	28.70	97.60ns	-2.59	11.88	1.42	13.90ns	-0.71	39.00ns	-1.27	850**	-1.73
EPN's (*Steinernema* sp.)	71.30	30.30	101.60ns	+1.40	12.75	1.55	14.30ns	+2.14	40.90*	+3.54	875ns	+1.16
Trichoderma harzianum	75.80	32.70	108.50**	+8.28	13.65	1.65	15.30**	+9.29	43.80**	+10.89	940**	-8.67
Neem cake	80.80	34.70	115.50**	+15.27	14.61	1.79	16.40**	+17.14	47.00**	+18.98	1000**	+15.61
Soyabean cake	77.80	33.20	111.00**	+10.78	13.99	1.71	15.70**	+12.14	45.50**	+15.19	970**	+12.14
C.D. at *P*=0.05	-	-	3.20	-	-	-	0.37	-	1.49	-	22.62	-
C.D. at *P*=0.01	-	-	4.44	-	-	-	0.52	-	2.09	-	31.40	-

Values are mean of five replicates
* significant over control at 5%:
** significant over control at 5% and 1%



T1 = Control (uninoculated untreated), T2 = *Meloidogyne incognita*, T3 = *Carbofuran*, T4 = *Paecilomyces lilacinus*, T5 = EPN's, T6 = *Trichoderma harzianum*, T7 = Neem cake, T8 = Soyabean cake

Bars with the same letters are non significant among the treatments according to Duncan's multiple range test ($P \leq 0.05$).

Fig. 5.0 Effect of treatments of biocontrol agents, oil cakes and carbofuran on the growth of eggplants.

weight, number of flowers and weight of fruits as compared to the plants grown in untreated soil (control).

4.8. Management of *Meloidogyne incognita* infecting eggplant by using biocontrol agents, oil-cakes and nematicide-carbofuran alone or in combination with *Steinernema* sp:-

The data presented in tables 9.1, 9.2 and figures 6.1, 6.2 revealed that *M. incognita* caused significant reduction in plant growth and increase in nematode multiplication as compared to un-inoculated and untreated plants. The reduction in various plant growth parameters such as plant length, dry weight, number of flowers and weight of fruits was recorded as 39.62%, 45.00%, 51.90% and 48.55% in comparison to control plants. The reproduction factor and number of galls/root system was recorded as 15.00 and 84.00 respectively.

However, the inoculation of eggplant with biocontrol agents viz., *Paecilomyces lilacinus, Trichoderma harzianum* and EPN's (*Steinernema* sp.) or soil amendments with oil-cakes (Neem cake and soyabean cake) or nematicide carbofuran significantly reduced the multiplication of *M. incognita* and the root galling caused by root-knot nematode which consequently increased the eggplant growth parameters in comparison to untreated and *M. incognita* inoculated plants.

Moreover, the highest improvement in plant growth parameters and best protection against *M. incognita* was obtained by application of carbofuran followed by *Paecilomyces lilacinus* , *T. harzianum* , Neem cake, *Steinernema* and soyabean cake. In the respective treatments ,the percentage reduction in plant length was recorded as 15.97%, 20.16%, 26.15%, 30.44%, 30.64% and 33.73% and dry weight as 18.57%, 22.14%, 29.29%, 32.14%, 32.86% and 39.29% and number of flowers as 23.29%, 28.60%, 33.67%, 32.97%, 38.73% and 44.30% and weight of fruits as 20.23%, 24.86%, 30.64%, 34.68 %, 35.84% and 41.62% in comparison to control plants. In the corresponding treatments the reproduction

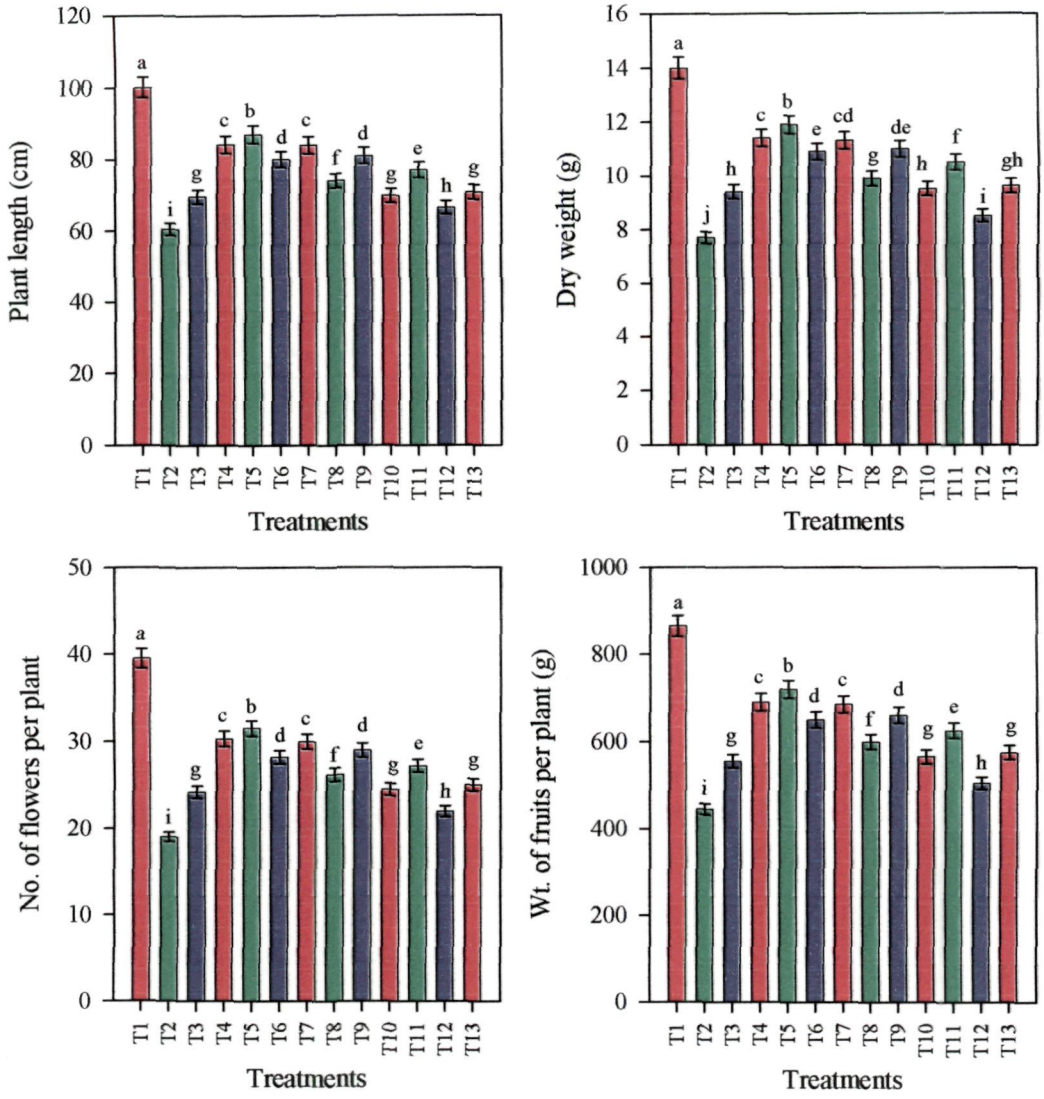
Table 9.1 Effect of biocontrol agents, oil cakes and nematicide -carbofuran alone or in combination with *Steinernema* sp. on the growth of eggplant infected with *M. incognita*.

Treatments	Plant length (cm)				Dry weight (g)				No. of flowers/ plant	% decrease over control	Weight of fruits /plant (g)	% decrease over control
	Shoot	Root	Total	% decrease over control	Shoot	Root	Total	% decrease over control				
Control (uninoculated & untreated)	70.3	29.9	100.20	-	12.49	1.51	14.00	-	39.5	-	865	-
***Meloidogyne incognita* (Mi)**	43.0	17.5	60.50**	-39.62	6.90	0.80	7.70**	-45.00	19.0**	-51.90	445**	-48.55
Mi + EPN's(*Steinernema* sp.)	49.7	19.8	69.50**	-30.64	8.44	0.96	9.40**	-32.86	24.2**	-38.73	555**	-35.84
Mi + Carbofuran	60.2	24.0	84.18**	-15.97	10.20	1.20	11.40**	-18.57	30.3**	-23.29	690**	-20.23
Mi + EPN's + Carbofuran	61.8	25.2	87.00**	-13.17	10.66	1.24	11.90**	-15.00	31.5**	-20.25	720**	-16.76
Mi + *Paecilomyces lilacinus*(Pl)	57.1	22.9	80.00**	-20.16	9.77	1.13	10.90**	-22.14	28.2**	-28.60	650**	-24.86
Mi + EPN's + Pl	59.8	24.2	84.00**	-16.17	10.12	1.18	11.30**	-19.29	30.0**	-24.05	685**	-20.81
Mi + Trichoderma harzianum(Th)	53.0	21.0	74.00**	-26.15	8.88	1.02	9.90**	-29.29	26.2**	-33.67	600**	-30.64
Mi + EPN's + Th	57.9	23.1	81.00**	-19.16	9.85	1.15	11.00**	-21.43	29.0**	-26.58	660**	-23.70
Mi + Neem cake (NC)	49.8	19.9	69.70**	-30.44	8.52	0.98	9.50**	-32.14	24.5**	-32.97	565**	-34.68
Mi + EPN's + NC	54.7	22.3	77.00**	-23.15	9.42	1.08	10.50**	-25.00	27.2**	-31.14	625**	-27.75
Mi + Soyabean cake (SC)	47.4	18.3	66.40**	-33.73	7.63	0.87	8.50**	-39.29	22.0**	-44.30	505**	-41.62
Mi + EPN's + SC	50.5	20.1	70.60**	-29.54	8.60	1.00	9.60**	-31.43	25.0**	-36.71	575**	-33.52
C.D. at *P*=0.05	-	-	2.80	-	-	-	0.30	-	0.90	-	23.54	-
C.D. at *P*=0.01	-	-	3.80	-	-	-	0.41	-	1.24	-	31.90	-

Values are mean of five replicates

* significant over control at 5%;

** significant over control at 5% and 1%



T1 = Control (uninoculated untreated), T2 = *Meloidogyne incognita*(Mi)
T3 = Mi + EPN's, T4 = Mi + Carbofuran, T5 = Mi + EPN's + Carbofuran,
T6 = Mi + *Paecilomyces lilacinus*(Pl), T7 = Mi + EPN's + Pl,
T8 = Mi + *Trichoderma harzianum* (Th),
T9 = Mi + EPN's + Th, T10 = Mi + Neem cake(NC),
T11 = Mi + EPN's + NC, T12 = Mi + Soyabean cake(SC),
T13 = Mi + EPN's + SC

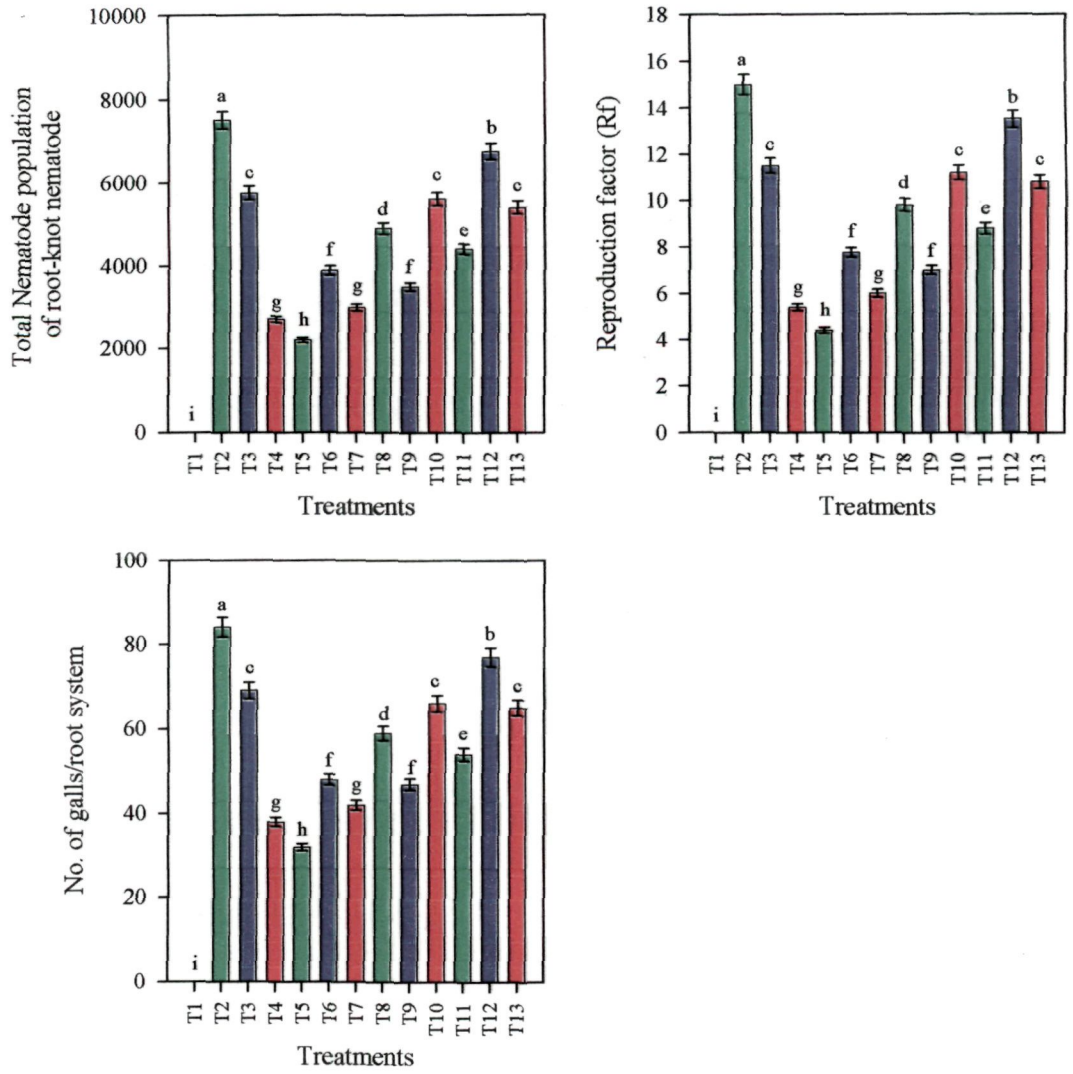
Bars with the same letters are non significant among the treatments according to Duncan's multiple range test ($P \leq 0.05$).

Fig. 6.1 Effect of biocontrol agents, oil cakes and carbofuran alone or in combination with *Steinernema* sp. on the growth of eggplants infected with *M. incognita*.

Table 9.2 Effect of biocontrol agents, oil cakes and carbofuran alone or in combination with *Steinernema* sp. on the multiplication of *M. incognita* infecting eggplants.

Treatments	Nematode population of RKN / 500g soil				No. of galls/ root system
	Females	Larvae	Total	R.f.	
Control (uninoculated & untreated)	–	–	–	–	
***Meloidogyne incognita* (Mi)**	230	7270	7500**	15.00**	84.00**
Mi + EPN's	175	5725	5750**	11.50**	69.00**
Mi + Carbofuran	128	2572	2700**	5.40**	38.00**
Mi + EPN's + Carbofuran	117	2083	2200**	4.40**	32.00**
Mi + *Paecilomyces lilacinus* (Pl)	145	3755	3880**	7.76**	48.00**
Mi + EPN's + Pl	134	2866	3000**	6.00**	42.00**
Mi + *Trichoderma harzianum (Th)*	149	4751	4900**	9.80**	59.00**
Mi + EPN's + Th	140	3360	3500**	7.00**	47.00**
Mi + Neem cake (NC)	170	5430	5600**	11.20**	66.00**
Mi + EPN's + NC	152	4248	4400**	8.80**	54.00**
Mi + Soyabean cake (SC)	195	6555	6750**	13.50**	77.00**
Mi + EPN's + SC	166	5234	5400**	10.80**	65.00**
C.D. at *P*=0.05	–	–	389.77	0.77	4.53
C.D. at *P*=0.01	–	–	526.84	1.05	6.13

Values are mean of five replicates
* significant over control at 5%;
** significant over control at 5% and 1%



T1 = Control (uninoculated untreated), T2 = *Meloidogyne incognita*(Mi),
T3 = Mi + EPN's, T4 = Mi + Carbofuran, T5 = Mi + EPN's + Carbofuran,
T6 = Mi + *Paecilomyces lilacinus*(Pl), T7 = Mi + EPN's + Pl,
T8 = Mi + *Trichoderma harzianum*(Th),
T9 = Mi + EPN's + Th, T10 = Mi + Neem cake(NC),
T11 = Mi + EPN's + NC, T12 = Mi + Soyabean cake(SC),
T13 = Mi + EPN's + SC

Bars with the same letters are non significant among the treatments according to Duncan's multiple range test ($P \leq 0.05$).

Fig. 6.2 Effect of biocontrol agents, oil cakes and carbofuran alone or in combination with *Steinernema* sp. on the multiplication of *M. incognita* infecting eggplant.

factor was recorded as 5.40, 7.76, 9.80, 11.20, 11.50, 13.50 and number of galls per root system was recorded as 38, 48, 59, 66, 69 and 77.

Moreover, the combined application of EPN's with either of the fungal bio-control agents (*P. lilacinus* and *T.harzianum*), oil cakes (neem & soyabean cakes) or carbofuran showed a greater significant reduction in reproduction factor and root galling caused by *M. incognita* which consequently increased the eggplant growth parameters in comparison to those plants individually treated with *P. lilacinus*, *T. harzianum*, *Steinernema* sp., neemcake, soyabean cake and carbofuran. The highest improvement in plant growth parameters and best protection against *M.incognita* was obtained by the integration of EPN's with carbofuran followed by EPN's with *P.lilacinus*, EPN's with *T. harzianum*,EPN's with neem cake and EPN's with soyabean cake. In the corresponding treatments the percentage reduction in plant length was recorded as 13.17%,16.17%,19.16%,23.15% and 29.54% and dry weight as 15.00% ,19.29%,21.43%,25.00% and 31.43% and number of flowers as 20.25%,24.05%,26.58%,31.14% and 36.71% and weight of fruits as 16.76%,20.81,23.70%,27.75% and 33.52% in comparison to control plants. In the corresponding treatments the reproduction factor were recorded as 4.40, 6.00, 7.00, 8.80, 10.80 and number of galls per root system was recorded as 32,42,47,54 and 65.The significant variation in plant growth parameters (except number of flowers per plant between the treatments of *M. incognita* + EPN's+ *P. lilacinus* and *M. incognita* + *P. lilacinus*), reproduction factor and number of galls were also recorded among these treatments except between the treatments of *M. incognita* + EPN's.

Chapter 5

DISCUSSION

CHAPTER 5

DISCUSSION

Knowledge of the biodiversity and geographical distribution of entomopathogenic nematodes is in its infancy, even though a number of surveys have been carried out, covering parts of every continent, most of the world still remains unexplored. In many of these surveys, major questions regarding the identification of the nematodes isolated and the methods used to recover them cannot be resolved. Thus, much survey has not been made for entomopathogenic nematodes, though the habitats are continuously under threat (Hominick *et al.,* 1996). Loss of habitats means loss of biodiversity and eventually loss of potential useful species. Thus, entomopathogenic nematodes are lost ever before their actual size is known and identification is done. Keeping in view, the above facts and vast and diverse agroclimatic conditions, the present work has been carried out to survey various localities of Aligarh (U.P.), Pulwama (J&K) and Shopian (J&K) districts of India aimed to collect indigenous entomopathogenic nematodes adapted to different climates for possible use in biological control regimes. Nematodes of the families Steinernematidae and Heterorhabditidae have characteristics that make them extremely useful as biological control agents.

Numerous surveys have been conducted worldwide, resulting in the isolation of many species and strains, Australia (Akhurst and Bedding, 1986), North America (Akhurst and Brooks, 1984; Poinar *et al.*, 1987 Mracek and Webster, 1993; Rueda *et al.,* 1993; Nguyen and Smart, 1994), Puerto Rico(Roman and Beavers, 1982), South America (Doucet, 1986 Doucet and Doucet, 1990, Nguyen and Smart, 1990; Stock, 1995), Hawaii (Hara *et al.*, 1991), Europe (Mracek, 1980; Deseo *et al.*, 1984; Husberg *et al.*, 1988; Hominick and Briscoe, 1990), Griffin *et al.*, 1991, Africa (Shamseldean and Abd- Elgawad, 1994), India (Poinar *et al.*, 1992), Srilanka (Amarsinghe *et al.,* 1994), Asia (Zhang *et al.,* 1992;

Choa *et al.,* 1995) and Japan (Kushida *et al.*, 1987; Mamiya and Ogura, 1989; Kimura and Ishibashi, 1991). In the broadest sense, entomopathogenic nematodes are widespread. The only continent where they have not been found is Antarctica (Griffin *et al.*, 1990). This conclusion arises from the results of a number of surveys but more precise statements are difficult because identification is frequently stopped at the generic level. Also, literature suggests that Steinernematids dominate in cooler, temperate soils while Heterorhabditids dominate in tropical conditions, but this broad generalization has recently been called into question (Amarsinghe *et al.*, 1994). Present study reveals that both in tropical (Aligarh district of U.P.) and temperate (Pulwama and Shopian districts of Kashmir) soils, Steinernematids dominate.

In India, research on entomopathogenic nematodes (EPN's) is being carried out at different centers which has led to the isolation of several EPN strains, and only a few have been identified while several strains are yet in queue waiting for identification (Ganguly, 2003). Thus, there is need to explore the native species and strains of EPN's which could perform well under given set of environmental conditions and could provide better and long lasting control. The present survey indicates that the Indian environment has rich entomopathogenic nematode fauna and each nematode has characteristic distribution pattern. Thus, there is need of further surveys to be undertaken to know the actual size and distribution of Indian entomopathogenic nematode fauna before it is lost due to habitat destructions. With the advancement of exploration of EPN's, research towards safe and effective control methods would be warranted.

In the present study the inoculations of either *Heterorhabditis* sp. or *Steinernema* sp. at different inoculum levels (50, 500, 1000, 2500,5000,10,000 and 20000 J3/500 g soil) on the eggplants did not significantly affect the growth of eggplants. This finding was found to be in disagreement with the finding of Fallon *et al.* (2002). Fallon *et al.* (2002) observed the increased plant growth in tomato plants by *S. riobrave* treatment. The difference in the findings might be due to that

entomopathogenic nematodes tend to die rapidly after their alone application to soil (Curran,) and/or different host, EPN species/race used in the experiments.

Root knot nematodes are the most prevalent and important group of plant parasitic nematodes occurring throughout the world but found more frequently and in greater numbers in areas having tropical climate. Root knot nematodes not only deprive nutrients of infected plants but also reduce the quantity and market value by affecting the quality of the fruit. Successful and economical management of root knot nematodes in view of worldwide distribution and extensive host range particularly of the four major species has been always a necessity of vegetable growers across the world. Chemical nematicides, which were effective to combat nematode pests and in use for the management for a long time, have been found to cause pollution. Increasing awareness of environmental and human health concerns associated with chemical nematicides and removal of several efficacious products from the world market in recent years provide impetus for a search of environmentally compatible products for nematodes management. Biological control of root knot nematodes, as for other pathogens, parasites and pests is currently in focus and is thrust area, which is receiving attention in almost all parts of the world. Biological control in addition to being cheap and effective is the safest in terms of environmental consideration and wants relatively less technical skill for their application. Keeping in view these aspects, the present study has focused attention to search effective biocontrol agents, which can be developed as biopesticides.

The use of entomopathogenic nematodes has been suggested as one possible alternative in the management of plant parasitic nematodes on vegetable crops. Application of EPN's to the soil significantly reduced abundance, diversity and maturity of the nematode community by reducing of genera and abundance of plant parasitic nematodes (Somasekhar *et al.*, 2002). During the last two decades, studies have reported an antagonistic interaction between entomopathogenic nematodes and plant parasitic nematodes. Soil in sealed containers where

entomopathogenic nematodes had been added had fewer plant parasitic nematodes than the control soil (Ishibashi and Kondo, 1986). Additional research has documented plant-parasitic nematode suppression by entomopathogenic nematodes in greenhouse and field studies (Smitley *et al.*, 1992; Gouge *et al.*, 1994; Grewal *et al.*, 1997, 1999; Perry *et al.*, 1998; Samaliev *et al.*, 2000; Lewis *et al.*, 2001). EPN's had suppressed a number of potentially important plant parasitic nematodes, including *Aphelenchoides rhytium* (Hu *et al.*, 1999), *Belonolaimus longicaudatus* and *Criconemella* sp. (Grewal *et al.*, 1997), *Globodera rostochiensis* (Perry *et al.*, 1998), *Haplolaimus* sp. (Perez and Lewis, 2006), *Meloidogyne* spp. (Bird and Bird, 1986; Ishibashi and Choi, 1991; Gouge *et al.*, 1994; Hu *et al.*, 1995; Ishibashi and Matsunaga, 1995; Lewis *et al.*, 2001; Fallon *et al.*, 2002 and Perez and Lewis, 2002 and 2004), *Mesocriconema xenoplax* Nyczepir *et al.*, 2004 , *Pratylenchus coffeae* (Ishibashi and Matsunaga, 1995), and *Tylenchorhynchus* spp. (Smitley *et al.*, 1992). Overall, more positive reports of suppression with EPN's have been reported for *Meloidogyne* spp. than for other PPN species (Lewis and Grewal, 2005).

Keeping in view above facts of suppression of *Meloidogyne* spp. by EPN's, this study was carried out to assess the efficacy of native species and strains of EPN's isolated from different regions (U.P. and J and K) of India in the control of *Meloidogyne incognita* infecting eggplant.

The present study revealed that the application of *Steinernema* sp. at different concentrations (50, 500, 1000, 2500, 5000, 10,000 and 20,000 J3 / 500 g soil) in eggplant seedlings inoculated with *Meloidogyne incognita* showed that both lower (50,500) as well as higher (20,000) inoculum levels of *Steinernema* sp. were insignificant in the reduction of root knot nematode population and galls produced by *Meloidogyne incognita* and consequently does not improve plants growth parameters viz., plant length, dry weight, number of flowers and weight of fruits. These findings were also found to be in agreement with Fallon *et al.*, (2002) and Sherbiny *et al.* (2007). Fallon *et al.*, (2002) reported that *Steinernema* sp. did

not affect the growth or development of *M. javanica* infected tomatoes at higher (20,000 ij's i.e. 44 ij's/cm^3) concentration levels. Sherbiny *et al.*, (2007) reported that the application of both *Steinernema feltiae* and *Heterorhabditis bacteriophora* at low application rates (1000 to 8000 J3 / pot), almost had no effects on improving visual growth parameters and pod weights of common bean in treated plants as compared to *M. javanica*- infected ones. Our results revealed that only at the moderate inoculum levels (1000, 2500, 5000 J3 / 500g soil) of *Steinernema* sp. were significant in reduction / suppression of the reproduction factor and galls produced by *Meloidgyne incognita* which consequently improved the plant growth parameters viz., plant length, dry weight, number of flowers and weight of fruits. However, our findings are in disagreement with the findings of Lewis *et al.*, (2002). They reported that both the lower and higher rates of *S. riobrave* suppressed *M. incognita.* The reason for higher inoculum levels to be non-significant in improving the plant growth of *M. incognita* infected plants is due to greater numbers of ij's of *Steinernema* sp. entering the root and consequently increasing entry points for subsequent entry of root knot nematode. This behaviour might have resulted in increased *M. incognita* root penetration at higher ij application levels of *Steinernema* sp. and consequently result in negative impacts on root increase.

The suppressive effects of entomopathogenic nematodes on plant parasitic nematodes may be due to several factors. Bird and Bird (1986) demonstrated the attraction of *Steinernema glaseri* Steiner to tomato roots and suggested that suppression of plant parasitic nematodes by entomopathogenic nematodes may be due to competition between the two nematode groups for space. However, this mechanism does not explain the suppression of plant parasitic nematodes by application of dead EPN's observed in various studies. Ishibashi and Kondo (1986) attributed the suppressive effects of entomopathogenic nematoes to the increased density of predators resulting from the application of nematode biomass to the soil. However, based on the observations of suppressing populations of plant

parasitic nematodes by entomopathogenic nematodes in sterile soil, and the suppression of root penetration of *Meloidogyne incognita* by heat killed entomopathogenic nematodes, Grewal *et al.* (1999) suggested that behavioural response and increased natural enemies are unlikely to account for the entire effect observed in the field. More recent evidence (Grewal *et al.*, 1999; Hu *et al.*, 1999; Lewis *et al.*, 2001; Samaliev *et al.*, 2000; Jagdale *et al.*, 2002) attributes the suppressive effects to the production of allelochemicals by the entomopathogenic nematode-symbiotic bacteria complex. Nematicidal properties of metabolites of symbiotic bacteria *Xenorhabdus* spp. associated with *Steinernema* spp. have been demonstrated in several laboratory / greenhouse studies (Grewal *et al.*, 1999; Hu *et al.*, 1999; Samaliev *et al.,* 2000). EPN- associated bacteria, *Xenorhabdus* spp. or *Photorhabdus* spp., produce endotoxins composed of lipopolysaccharides that are toxic and could kill or affect in another way the evaluated stages (Dunphy and Webster, 1988). According to Hu *et al.*, 1995, 1996, 1999, the bacteria produce Stilbene and Indole metabolites that are nematicidal to a range of nematode species.

The literature so for available does not reveal a standard rate of EPN's or application method to suppress plant parasitic nematodes in field and greenhouse experiments. For example, one application of 5×10^6 or ten daily application of 5×10^5 *S. glaseri* ij/pot suppressed *M. javanica* on tomato in greenhouse experiments (Bird and Bird, 1986). In turf, Smitley *et al.* (1992) used a mixture of 2.47×10^9 *H. bacteriophora* and 2.08×10^9 *S. carpocapsae* ij/ ha, whereas Grewal *et al.* (1997) used a single application of 2.47×10^9 *S. riobrave* ij/ha and suppressed plant parasitic nematodes in field experiments. In the laboratory, a rate of 2.5×10^9 *S. feltiae* ij/ha applied simultaneously with *M. incognita* suppressed the latter on tomato seedlings grown in sterilized sand (Lewis *et al.*, 2001). Fallon *et al.* (2002) reported that there was no dose response effect on *M. javanica* root penetration by *S. feltiae* or *S. riobrave* or effect on egg production by *S. riobrave*

at the concentration of 100 ijs / cm^3. The application of EPN's does not always reduce plant parasitic nematode populations and the outcome of their interaction vary according to EPN species, the crop receiving the application and the method used to evaluate the impact on the plant parasitic nematode (Lewis and Grewal, 2006).

The simultaneous inoculation of *M. incognita* and either of the inoculum levels(1000,2500,5000,10,000) of the *Steinernema* sp. significantly improved the growth parameters of eggplants and reduce the root knot nematode reproduction factor and number of galls /root system as compared to *M. incognita* inoculated plants. However, on the other hand the sequential inoculation of *Steinernema* sp. at different inoculum levels (1000, 2500, 5000, 10,000 and 20,000) one week before or after inoculation of *M. incognita* showed the significant improvement in plant growth parameters and reduction in reproduction factor and number of galls /root system. These findings also showed that the sequential inoculation of *Steinernema* sp. at different inoculum levels (1000,2500, 5000,10,000 and 20,000) were more effective in the management of root-knot nematode than the concomitant inoculation.These findings were found to be in agreement with Lewis and Perez (2002); Grewal, (1997); Sasnarukkit *et al.*, (2002); Evan & Haydock, (1993). Lewis and Perez (2002) found that *S. riobravis* and *S. feltiae* applied at the rate of 25ijs/cm^2 before or after and *H. bacteriophora* applied before *M. incognita* were most efficient in suppression of penetration and egg production on tomatoes than the concomitant application. They also reported that pre-or post *M. incognita* infestation application of 25 ij/cm^2 of *H. bacteriophora, S. riobrave* or *S. feltiae* suppressed *M. incognita* on tomato plants grown in green house. Sasnarukkit *et al.* (2002) reported that antagonism was observed only when inoculation of root-knot nematode ij's was done after the application of EPN's. EPN's tend to die rapidly after their inundative application to soil (Curran 1993). The entomopathogenic nematodes dying and serving as substrate for the growth of their symbiotic bacteria resulted in the production of antagonistic metabolites. Evan and Haydock

(1993) also reported that the prior application of EPN's may lead to the production of nematicidal metabolites. Moreover, my results were in disagreement with the finding of Perez and Lewis (2004), who reported that not only pre-application of EPN's are effective but the application at the same time with root knot nematodes is equally effective.

Grewal *et al.* (1999) reported that root penetration of *M. incognita* was suppressed by application of dead or heat killed *S. riobrave* or *S. feltiae*, but found no effect when living nematodes were used. However, on the other hand Jagdale *et al.* (2002) reported that dead *S. carpocapsae* infective juveniles were as effective as live nematodes in controlling plant-parasitic nematodes. These findings are of practical importance because the use of dead EPN's may help in overcoming the difficulties in formulation, storage and transportation associated with the use of living systems for biological control of nematodes.

Thus our results are also in conformity with the previous studies that entomopathogenic nematodes can be used for the control of plant parasitic nematodes(Bird and Bird, 1986; Ishibashi and Kondo, 1986; Ishibashi and Choi, 1991; Kermarrec *et al.*, 1991; Smitley *et al.*, 1992; Gouge *et al.*, 1994; Matsunaga *et al.*, 1997; Grewal *et al.*, 1997;Perry *et al.*, 1998; Aalten and Gowen, 1998; Riegel *et al.*, 1998; Grewal *et al.*, 1999; Somasekhar *et al.*, 2000; Lewis *et al.*, 2001; Lewis and Perez, 2002; Jagdale *et al.*, 2002; Perez and Lewis, 2004; and Burelle and Lewis, 2004). Moreover, suppressive effect of EPN on root-knot nematodes depends upon application time and species (Lewis & Perez, 2002).

The results also revealed that when EPN's, *T. harzianum, P. lilacinus,* carbofuran and oil-cakes (Neem and soyabean) were applied individually to egg plants, only *T. harzianum* and oil-cakes (neem and soyabean) proved to be effective in enhancing the growth parameters such as plant length, dry weight, number of flowers and weight of fruits. The *T. harzianum* to be effective in enhancing the growth of egg plants is also in agreement with Pathak *et al.*, (2007)

who reported that *Trichoderma* spp. not only act as a bioagent for disease control but also enhances the growth of plants by providing soil nutrition in soluble forms for absorption by the roots resulting in better health of plants. *Trichoderma* spp. are known to solublize rock phosphate, metallic zinc, manganese, iron copper and also enhance nitrogen use efficiency in plants. The application of oil cakes (neem and soyabean) also enhances the growth of health plants seems likely due to the fact that organic matter present in soil is important in binding soil particles together, while the micro-organisms associated with organic matter have an additional cementing action. Organic matter therefore plays a major role in the formation and stabilization of soil aggregates, so that when it is added to soil it has effects on characteristics such as soil aeration, the infiltration rate of water into soil and soil water holding capacity. Improvement in plant growth may occur as a result of changes in the nutrient status and physical characteristics of soil, and plant damaged by pathogens may also respond to organic matter for the same reasons. It is well recognized that healthy plants growing under ideal environmental conditions are able to tolerate pathogen damages much better than thrifty plants.

Modern agricultural practices often require the application of multiple types of control agents to reduce pest and disease damages. These methodologies must be compatible and integrated in overall production systems. In the present study, the compatibility of EPN's (*Steinernema sp.*) was checked with fungus (*P. lilacinus* and *T. harzianum*), oil-cakes (neem and soyabean) and the nematicide carbofuran in controlling *M. incognita* in eggplants. Our attempts were to test the effectiveness of their different combinations against *M. incognita.*

The results of present study clearly indicated that the application of biocontrol agent's viz., *Paecilomyces lilacinus* and *Tricoderma harzianum* significantly reduced the reproduction factor and number of galls per root system which consequently improved the plant growth parameters such as plant length, dry weight, and number of flowers and weight of fruits in the egg plants

inoculated with *M. incognita*. These results are also in agreement with those of Shahzad and Ghaffar, 1989; Siddiqui and Mahmood, 1992; Siddiqui *et al.*, 2000; Haggag and Amin, 2001; Chaitali *et al.*, 2003; Raut and Patel, 2005; Haseeb and Kumar, 2006; who reported the use of fungal biocontrol agents viz. *Trichoderma harzianum* and *Paecilomyces lilacinus* to control root-knot disease on different plants. The efficacy of *P. lilacinus* against root-knot nematode has been reported by various workers, Zaki and Bhatti, 1990; Walia *et al.,* 1991; Anita and Vadivelu, 1997 and Zaki and Mahmood, 1999 in brinjal, chickpea, cowpea and betelvine, respectively. It is reported that *P. lilacinus* is capable of parasitizing nematode eggs and destroying the embryo. It also grows within the body of larvae and developing females resulting in their death (Jatala *et al.,* 1979). Earlier reports showed that the hatched out juveniles become incapacitated in the presence of fungal hyphae indicate a primarily, diffusible toxic effect rendering them subsequently vulnerable to colonization. It is possible that partial disintegration of vitelline layer may be due to exoenzyme production by the fungal hyphae, possibly involving the physiologically disorganizing factor, such as diffusible toxic metabolites. This disruption not only predisposes the egg to fungal infection by physical weakening of the shell, but also increase permeability, thus facilitating inward passage of fungal metabolites, both toxic and enzymatic. This exopathic effect might be enough to abort the reproductive process. Once a fungal hypha enters the egg, enzymatic dissolution of the chitin layer takes place (Okafor, 1976). It has been reported that mycelial proliferation on the nematode body results in probable biosynthesis of destructive metabolites endogenously. Therefore endogenous mycelial proliferation might support the lysis of egg-shall material. Later, hyphae penetrate the larval cuticle. This endopathic activity of the fungus causes total degeneration of the egg contents and leads to the ultimate mortality of the larvae. *P. lilacinus* isolates from silkworms yielded oxalic, dipicolinic and succinic acids and some unidentified amino acids as well as large amounts of D-mannitol (Domsch *et al.,* 1980). These chemicals might also be

responsible for the killing of nematodes. Saeed *et al.,* 1988 found that *P. lilacinus* does not produce aflatoxin and thus can be used safely on a large scale under field conditions.

Similarly, in the present study *Trichoderma harzianum* have effectively suppressed the reproduction factor and number of galls per root system produced by *M. incognita* which led to increase in the growth parameters viz., length, dry weight, number of flowers and weight of fruits in *M. incognita* infected egg plants. These results are also supported by Pathak *et al.* (2007) who reviewed the management of nematode and fungal diseases by *Trichoderma* spp.The activity of *Trichoderma* spp. against root knot nematode might be due to release of antibiotics like alkylpyrones, isonitriles, polyketides, peptibols, diketopiperzones, sesquiterpenes, steroids etc. which are harmful to pathogen and therefore inhibits the growth of pathogen (Elwad *et al.,* 1982, Lorito *et al.,* 1993), such mechanism is called antibiosis. *Trichoderma* spp. also produces antibiotic called trichodermin. Once *Trichoderma* is introduced in the soil either with treated seeds or mixed with farmyard,it grows so rapidly consuming essential nutrients from soil that the other soil inhabiting microorganisms fail to compete with the antagonist and finally die due to scarcity of food and space.

Another mechanism of biocontrol by *Trichoderma* is due to induced resistance in plants (Windham *et al.*, 1986). Many plant pathogens depend upon the production of pectolytic, cutinolytic and cellulolytic enzymes to infect host plants. Some of the *Trichoderma* strains produce serine protease that is capable to inactivate cell wall degrading enzymes of the pathogen and therefore reduce the ability of the pathogen to infect its host.

The present findings also revelaed that the ability of EPN's (*Steinernema* sp.) increased in presence of both *T. harzianum* and *P. lilacinus* in suppression of the reproduction factor and galls produced by *M. incognita* which consequently resulted in increase in plant growth parameters viz., plants length, dry weight, number of flowers and weight of fruits in *M. incognita* infected egg plants. These

findings are also in agreement with Luna *et al.*, 2005 and Noweer *et al.*, 2007. Luna *et al.* (2005) found that *P. lilacinus* and EPN's are compatible elements of integrated pest management. Noweer *et al.* (2007) also reported compatibility between EPN's and fungi. They reported highest tomato yield in treatment of combined application of EPN's and trapping fungi compared to separate treatments and control.

The present study also indicated that the ability of EPN's (*Steinernema* sp.) increased in suppression of reproduction factor and galls produced by *M. incognita* which consequently improved the growth parameters in presence of oil cakes of neem and soyabean. There is lack of literature regarding the combined application of EPN's and oilcakes in controlling *M. incognita.*

Materials with a high phenolic content are often used as soil amendments for nematode control and the results of several studies have suggested that these materials can act by altering the attractiveness of roots to nematodes. For example Sitaramiah and Singh (1974) observed that tomato seedlings raised in soil amended with neem (*Azadirchta indica*) oil-cakes showed a reduced level of infection by *Meloidogyne javanica* when compared with non-amended soil. A similar observation was made by Singh *et al.* (1983), who found that roots growing from seeds coated with a finely ground preparation of various oil-cakes were not penetrated by juveniles of *M. incognita.* The concentration of phenolic compounds in roots invariably increased in plants grown in association with oil-cakes and other materials containing phenols (Alam *et al.,* 1997; Sitaramaiah, Singh, 1978; Singh *et al.,* 1983) and it was these compounds that were thought to have been responsible for the reduced attraction of roots to nematodes.

However, evidence to support this hypothesis is based mainly on the correlation that has been observed between the presence of phenolics in plant tissue and the degree of resistance of the plant to the nematodes (Giebel, 1974). Since others believe there is no concrete evidence for the involvement of simple, pre-formed phenolic compounds or their oxidation products in the incompatibility

of plants to nematodes (Kaplan and Keen, 1980), the actual mechanism by which amendments containing phenols alter plant resistance requires further elucidation.

It may also be due to the reason that organic materials contribute to enhanced biological activities against the target pathogen by providing the needed nutrients for the initial growth of the biocontrol agents in soil and may be used as carriers to facilitate distribution. Some organic materials contain nematoxic chemicals and when these materials are used as soil amendments, the nematicidal principal is released into soil and is directly responsible for the suppression of RKN population and did not affect the efficacy of EPN's. Neem is perhaps the best documented example of such a material. The commonly occurring chemicals in neem e.g. azadirachtin, kaempferol, nimbin, nimbidin, nimbidic acid, querctin and thionemone are thought to be responsible for the management of root knot nematode. Neem is the best known example of an organic amendment that acts by releasing pre-formed nematicidal substances in soil. Neem oil cake having nematicidal effects is the subject of several reviews for e.g, Sing and Sitaramiah, 1996, 1971; Egunjobi and Afolami, 1976; Sitaramiah and Singh, 1978; Vijayalakshmi and Prasad, 1979; Bhatttacharya and Goswami, 1987, and it appears that limnoids present in neem oil cakes is directly responsible for this effect.

Moreover, the volatile fatty acids (formic, acetic, propionic and butyric), ammonia, formaldehyde, hydrogen sulphide, phenols and amino acids that are released during the decomposition of oil cakes have been reported toxic to nematodes (Parvath Reddy *et al.,* 1975; Alam *et al.*, 1978; Khan *et al.*, 1974).

During the present investigation it was also found that the efficacy of EPN's (*Steinernema* sp.) increased in suppression of reproduction factor and galls produced by *M. incognita,* which consequently improved the plant growth parameters when applied in combination with nematicide carbofuran. The compatibility of *Sterinernema* sp. with the nematicides was found to be in agreement with Singh & Bardhan (1974), Roa *et al.* (1975), Das & Divakar

(1987), Hussaini *et al.* (2001) and Easwaramoothy & Sankaranarayanan (2003). Easwaramoorthy & Sankaranarayanan (2003) reported that the *S. glaseri* was compatible with carbofuran, phorate, quinalphos and aldrin. Hussaini *et al.*, 2001 reported that isolates of both *Steinernema* sp. and *H. indica* were compatible with pesticide and could be used for field application or could be used safely in pesticide treated field to enhance the mortality of the pests. Sherbiny *et al.* (2007), who reported that nematicide cadusafos 10 G provided the maximum *M. javanica* reduction and the best plant performance in comparison to *Sterinernema feltiae* and *Heterorhabditis bacteriophora.* They also concluded that EPN's as biocontrol agents are not acceptable alternative to condusafos 10G in controlling *M. javanica* on common bean.

Chapter 6

SUMMARY

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As much of the world has not been surveyed for entomopathogenic nematodes and habitats are continuously under threat. The entomopathogenic nematodes are lost even before their actual size is known. Keeping in view the vast and diverse agro-climatic regions in our country, the present work was carried out and survey of various regions of Aligarh (U.P.), Pulwama (J & K) and Shopian (J and K) districts of India was aimed at collecting indigenous EPN's adapted to various climates for its possible use in biological control. Therefore ,it led a need to explore the native species and strains of EPN's which could perform well under given set of environmental conditions and could provide better and long lasting control strategy. In all the three regions surveyed, rich frequency of occurrence of EPN's was reported. Various other workers were also of the view that the Indian soil is rich in EPN diversity and distribution.

Biocontrol of plant pathogens has an edge over other control strategies because of the fact of their being environmentally safe as compared to environmental risks and health hazards involved in the use of chemical pesticides. Biological control mainly deals with the application of bioagents or by stimulation of natural enemies of the nematodes like fungi, bacteria and nematodes in the soil. Due to prevalence of occurrence of root knot nematode in Aligarh and positive reports of suppression of root knot nematode by EPN's by various authors, the EPN's isolated were used to assess their efficacy in controlling root knot nematode in brinjal occurring at Aligarh.

No significant variation in plant growth parameters (plant length, dry weight, number of flowers and weight of fruits) was recorded in the plants inoculated with different inoculum levels (50,500,1000,2500,5000,10,000,20,000J3/500g soil) of EPN's viz., *Steinernema* sp. and *Heterorhabditis* sp. as compared to uninoculated plants.

The simultaneous inoculation of *Meloidogyne incognita* and either of the inoculum levels viz., 1000, 2500, 5000 and 10,000 J3/500g soil of *Steinernema* sp. significantly reduced the reproduction factor and number of galls/root system which consequentally reduced the damage in terms of plant growth parameters viz., plant length, dry weight, number of flowers and weight of fruits. The inoculation of *Steinernema* sp. at lower(50,500) and higher (20,000) inoculum levels didn't significantly improve the growth parameters and reduce the reproduction factor and number of galls per root system as compared to the plants inoculated with *M. incognita* individually.

However, on the other hand, the sequential inoculation of *Steinernema* sp. at different inoculum levels(1000,2500,5000,10,000 and 20,000) one week before or after inoculation of *M. incognita* showed the significant improvement in plant growth parameters and reduction in reproduction factor and number of galls /root system .These findings also showed that the sequential inoculation of *Steinernema* sp. at different inoculum levels (1000,2500,5000,10,000 and 20,000) is more effective in the management of *M. incognita* than the concomitant inoculation.

The results also revealed that when EPN's, *T. harzianum*, *P. lilacinus*, carbofuran and oil-cakes (neem and soyabean) were applied individually to eggplants, only *T. harzianum* and oil cakes(neem and soyabean) proved to be significantly effective in enhancing the growth parameters such as plant length, dry weight, number of flowers and weight of fruits.

The inoculation of eggplants with biocontrol agents viz., *P. lilacinus*, *Trichoderma harzianum* and EPN's (*Steinernema* sp.) or soil amendments with oil cakes (neem and soyabean cake) or carbofuran significantly reduced the multiplication of *M. incognita* and the root galling caused by root-knot nematode which consequently increased the eggplant growth parameters such as plant length, dry weight, number of flowers and weight of fruits in comparison to untreated and *M. incognita* inoculated plants. However, the highest improvement in plant growth parameters and best protection against *M. incognita*

was obtained by application of carbofuran followed by *P. lilacinus*, *T. harzianum*, neem cake, *Steinernema* sp. and soyabean cake.

Moreover, the combined application of EPN's with either of the fungal biocontrol agents (*P. lilacinus* and *T. harzianum*), oilcakes (neem and soyabean cakes) or carbofuran showed a greater significant reduction in the reproduction factor and root galling caused by *M. incognita* which consequentaly increased the eggplant growth parameters in comparison to those plants treated individually with *P. lilacinus, T. harzianum,* neem cake, soyabean cake, carbofuran *and Steinernema* sp. The highest improvement in plant growth parameters and best protection against *M. incognita* was obtained by the integration of EPN's with carbofuran followed by EPN's with *P. lilacinus*, EPN's with *T. harzianum*, EPN's with neem cake and EPN's with soyabean cake. The significant variations in plant growth parameters (except number of flowers per plant between the treatments of EPN+ *P. lilacinus* and *P. lilacinus* alone), reproduction factor and number of galls were also recorded among these treatments except between the treatment of neem cake and EPN's.

Chapter 7

REFERENCES

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